HELDER CRAVO DA COSTA

CARACTERIZAÇÃO DA ORIGEM DAS FIBRAS IMUNORREATIVAS AO HORMÔNIO CONCENTRADOR DE MELANINA NA LÂMINA INTERNA DA EMINÊNCIA MEDIANA E NA HIPÓFISE POSTERIOR DURANTE A LACTAÇÃO EM RATAS LONG-EVANS (*RATTUS NORVEGICUS*)

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Morfofuncionais do Instituto de Ciências Biomédicas da Universidade de São Paulo, para obtenção do Título de Mestre em Ciências.

Área de Concentração: Ciências Morfofuncionais.

Orientador: Prof. Dr. Jackson Cioni Bittencourt.

Versão original.

São Paulo
2013

RESUMO

Costa, HC. Caracterização da origem das fibras imunorreativas ao hormônio concentrador de melanina na lâmina interna da eminência mediana e na hipófise posterior durante a lactação em ratas Long-Evans (*rattus norvegicus*). [dissertação (Mestrado em Ciências Morfofuncionais)]- Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo; 2013.

Introdução: Em ratos, encontramos a expressão de RNAm do prépró-hormônio concentrador de melanina (ppMCH) e o peptídeo MCH principalmente nas áreas hipotalâmica lateral e incerto hipotalâmica. Entretanto, durante a lactação novos sítios de expressão do ppMCH e de imunorreatividade ao MCH (MCH-ir) foram identificados, como a parte ventral da área pré-óptica medial (MPOA), ocorrendo o máximo de sua expressão no término da lactação (entre os dias 19º e 21º), e sem colocalização entre os neurônios produtores de ocitocina (OT) e de MCH no hipotálamo. Entretanto, também já foi demonstrado que o MCH induz a secreção de ocitocina na hipófise posterior (PPit) de ratos machos. Além disso, as fibras MCH-érgicas e OT-érgicas transitam pela lâmina interna da eminência mediana (MEi), mas não se conhece a origem das fibras MCH-ir e nem a sua possível relação com as fibras OT-érgicas. **Objetivos:** 1) descrever a origem das fibras imunorreativas ao MCH na MEi e na PPit em ratas lactantes; 2) descrever através da microscopia óptica as fibras MCH-érgicas e OT-érgicas e as relações entre elas na passagem pela MEi em direção à PPit em ratas lactantes; 3) quantificar o peptídeo ppMCH e OT na PPit em ratas lactantes. **Material e Métodos:** utilizamos ratas da linhagem Long-Evans, e os seguintes métodos: 1) a microscopia confocal para caracterizar as fibras MCH-ir e OT-ir que estão na MEi e na PPit; 2) a injeção de traçador neuronal retrógrado fluorescente, o *Fluoro-gold®* (FG) via intravascular e a combinação dos métodos de hibridização *in situ* e imuno-histoquímica para identificar as possíveis células duplamente marcadas (FG + RNAm ppMCH) no hipotálamo; 3) utilizamos a técnica de *Western Blotting* para quantificar o peptídeo ppMCH e OT na PPit. **Resultados:** 1) foi evidenciado, pela primeira vez, que há uma proximidade entre as fibras MCH-ir e OT-ir, 2) evidenciamos, também pela primeira vez, a colocalização de neurônios retrogradamente marcados com FG e que expressam RNAm do ppMCH na MPOA e na porção anterior do núcleo paraventricular do hipotálamo (PVHa), 3) demonstramos que há um aumento do peptídeo MCH e uma diminuição da OT na PPit no 19º dia de lactação. **Conclusão:** os dados demonstram que o surgimento da plasticidade neuronal para expressão do ppMCH na MPOA, a proximidade das fibras MCH-ir e OT-ir na MEi e a maior quantidade de MCH e menor de OT na PPit fazem parte de um controle neuroendócrino para o término do período de lactação e o comportamento maternal.

Palavras chaves: Ocitocina. Comportamento maternal. Hipófise. Hipotálamo. Fluoro-gold. Área pré-óptica medial.

ABSTRACT

Costa, HC. Characterization of the origin of melanin-concentrating hormone immunoreactive fibers in the internal layer of the median eminence and the posterior hypophysis during the lactation period in Long-Evans rats (*Rattus norvegicus*). [dissertation (Masters thesis in Science)] - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2013.

Introduction: It has been described that the expression of prepro-melanin-concentrating hormone (ppMCH) mRNA and MCH peptide are mainly localized in the lateral hypothalamic and in the incerto hypothalamic areas of the rat. However, during lactation new sites of ppMCH mRNA expression and MCH immunoreactivity (MCH-ir) were identified in the ventral part of the medial preoptic area (MPOA), with its peak at the end of lactation period (between the 19^{th} and 21^{st} days), besides, there is not colocalization between MCH and oxytocin-producing neurons (OT) in the hypothalamus. However, it has also been demonstrated that the MCH induces secretion of oxytocin in the posterior hypophysis (PPit) in male rats. Furthermore, MCH-ir and OT—ir fibers are described by passing in the internal layer of the median eminence (MEi). However, the origin of the MCH-ir fibers and its possible relationship with the OT-ir fibers are not known. **Objectives**: 1) describe the origin of the MCH immunoreactive fibers in the MEi and PPit in lactating rats, 2) describe through the optical microscopy the MCH-ir and OT-ir fibers and the relationship between them in passing by MEi toward PPit in lactating rats, 3) quantify the amount of ppMCH and OT in the PPit of lactating rats. **Material and Methods**: we have used Long-Evans rats, and the methods were: 1) confocal microscopy to characterize the relationship between MCH-ir and OT-ir fibers that are in the MEi and PPit, 2) injection of a fluorescent retrograde neuronal tracer, Fluoro-Gold ® (FG) intravascularly to identify possible sites of double-labeled cells (FG + ppMCH mRNA) in the hypothalamus by using a combination of methods of *in situ* hybridization and immunohistochemistry; 3) the technique of Western blotting was used to quantify ppMCH and OT in PPit. **Results**: 1) it was demonstrated for the first time, that there is a closeness between the MCH-ir and OT-ir fibers in the MEi, 2) it was evidenced also for the first time, the colocalization of ppMCH mRNA-producing neurons and FG in the MPOA and in the anterior portion of the paraventricular nucleus of the hypothalamus (PVHA), 3) there is an increase of ppMCH in the PPit, but a decrease of OT amount on the 19^{th} day of lactation. **Conclusion**: the data found in this work allow us to suggest that there is a neuronal plasticity mechanism in the MPOA of the MCH-producing neurons and, the proximity of MCH-ir and OT-ir fibers in the MEi, and that at the same time an increase of MCH-ir and a decrease of OT-ir in the PPit are part of a neuroendocrinological control that would work at the final stages of lactation and maternal behavior.

Keywords: Oxytocin. Maternal behavior. Hypophysis. Hypothalamus. Fluoro-Gold. Medial preoptic area

INTRODUÇÃO

O hormônio concentrador de melanina (MCH) tem sido muito estudado nos últimos anos como um dos principais neuromoduladores do comportamento alimentar em mamíferos (Elmquist, 2001; Elmquist et al., 1999; Niswender et al., 2004; Sawchenko, 1998; Smith, Grove, 2002; Woods, D'Alessio, 2008).

No entanto, outras funções podem ser atribuídas ao MCH, principalmente pelo fato de ser expresso essencialmente no hipotálamo. Provavelmente é um regulador de respostas endócrinas, autonômicas e comportamentais que garante a homeostasia do indivíduo, sua sobrevivência e, portanto, a manutenção da espécie.

Algumas características inerentes aos mamíferos e de suma importância para a sobrevivência da espécie estão nos períodos de gestação e de lactação. A lactação é um estado fisiológico natural posterior ao parto, caracterizado pela alta demanda energética, pela inibição da função ovariana, dentre outras alterações fisiológicas e comportamentais da fêmea requeridas pelas circunstâncias (Numan, 2006).

Estudos como o de Knollema et al. (1992) e Rondini et al. (2010) colocam o MCH como um possível regulador envolvido no processo de lactação, uma vez que relataram que ocorre a expressão do RNAm do pré-pró-hormônio concentrador de melanina (ppMCH) e a presença do peptídeo MCH, principalmente na parte ventral da área pré-óptica medial (MPOA) somente no período lactante. Foi relatado, também, que o pico dessa expressão ocorre no término da lactação (entre os dias 19º e 21º). A MPOA está localizada na região rostral do hipotálamo e com evidências muito robustas de seu envolvimento no comportamento maternal (Numan, Stolzenberg, 2009).

Um hormônio que tem seu papel há muito tempo conhecido na reprodução, principalmente durante o parto e na lactação é a ocitocina (OT). A OT é um hormônio hipotalâmico produzido e transportado por neurônios neurossecretores para a hipófise posterior (PPit) onde é liberado. Esses neurônios neurossecretores antes de chegarem à PPit passam pela eminência mediana (ME). Estrutura essa que é dividida em lâmina interna e externa, onde encontramos principalmente axônios e terminais de axônios muito próximos aos capilares sanguíneos, estabelecendo uma região de contato neuro-humoral entre o hipotálamo e a hipófise. É descrito na literatura a presença na lâmina interna da ME (MEi), de fibras imunorreativas ao MCH (Bittencourt et al., 1992) e também de fibras imunorreativas à OT (Swanson, 1987). Essa associação entre fibras MCH-érgicas e OT-érgicas sugere uma relação entre o MCH e a OT e que o MCH deva ter alguma função no término do período de lactação.

CONCLUSÃO

De acordo com os resultados obtidos, e frente aos objetivos propostos, podemos concluir que:

a) ocorre a justaposição entre as fibras MCH-ir e OT-ir na MEi e na PPit em ratas Long-Evans no 19º dia de lactação;

b) há um aumento da densidade de fibras MCH-ir na MEi e PPit no 19º dia de lactação;

c) existe uma relação inversa entre a quantidade do peptídeo ppMCH e OT na PPit no 19º dia de lactação;

d) a origem das fibras MCH-ir que transitam pela MEi em direção a PPit na fase de lactação está na MPOA;

e) existe o surgimento de neurônios na MPOA que expressam o RNAm do ppMCH e que são retrogradamente marcados no 19º dia de lactação, e que não aparecem fora desse período.

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