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Research report

Dephosphorylation/inactivation of tyrosine hydroxylase at the median eminence of the hypothalamus is required for suckling-induced prolactin and adrenocorticotrop hormone responses

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ABSTRACT

We have recently found that dopamine (DA) released from terminals of the hypothalamic neuroendocrine dopamine (NEDA) neurons plays a role not only in prolactin (PRL), but also in adrenocorticotrop hormone (ACTH) secretion, without having any influence on α-melanocyte-stimulating hormone (α-MSH) release in lactating dams. The aim of our present studies was to further investigate this DAerg regulation of ACTH using consecutively applied physiological stimulation (suckling) and pharmacological inhibition of the rate-limiting enzyme of DA synthesis (tyrosine hydroxylase, TH) by α-methyl-p-tyrosine (α-MpT) that acutely affect secretion of these pituitary hormones during lactation. Following 4 h separation period, two experimental groups were formed. In the first group, lactating rats were assembled with their litters for 60 min prior to α-MpT. In the second group, the α-MpT was injected first and 60 min later suckling stimulus was applied. Plasma samples were taken in every 15 min during the 90 min experimental period. Concentrations of plasma PRL, ACTH and α-MSH were measured by specific RIAs. Both stimuli applied in the first sequence, significantly elevated plasma PRL and ACTH levels in separated lactating dams, without having any effect on α-MSH secretion. Suckling applied in the first sequence was able to block the α-MpT-induced elevation of ACTH secretion, while PRL response was also significantly attenuated. α-MpT pretreatment prevented both PRL and ACTH responses to suckling stimulus. Investigating the dephosphorylation/inactivation of TH in the arcuate nucleus-ME (TIDA) regions, no pTH-immunoreactive perikarya or terminals can be found in continuously suckled dams. In contrast, after 4 h separation of the mothers from their litters, pTH-immunoreactivity can be clearly visualized in the external zone of ME. In α-MpT pretreated mothers following 4 h separation no pTH positive terminals are visible. No changes in the TH immunostaining can be observed in any of these experimental groups. In conclusion, dephosphorylation/inactivation of TH (the rate-limiting enzyme of the DA biosynthesis) in NEDA neurons is required for suckling-induced PRL and ACTH responses.

1. Introduction

It is well known that the secretion of both prolactin- (PRL) and pro-opiomelanocortin (POMC) derived α-melanocyte-stimulating hormone (α-MSH), from the anterior (AL) and the intermediate lobe (IL), respectively, is under a tonic inhibition exerted by the hypothalamic neuroendocrine dopaminergic (NEDA) neurons in rats. Cell bodies of NEDA neurons located in the arcuate nucleus can be divided into three groups based upon the rostro-caudal subdivisions of their cell bodies in the hypothalamus as well as the difference in projection sites of them. Perikarya of tuberoinfundibular DA (TIDA) neurons locate in the caudal portion of the arcuate nucleus [9], and terminate at the external zone of median eminence (ME). Tuberohypophysial DA (THDA) neurons originate from the rostral portion of the arcuate nucleus, passing through the internal zone of ME and project to both the neural (NL)-, and the intermediate lobe (IL) [3]. In addition, a third subgroup of NEDA neurons locate in the rostral periventricular region (PHDA) of the hypothalamus and their axons run through the ME like axons of THDA neurons, but they exclusively innervate cells found in the IL [12]. Therefore, anatomically distinct subgroups of NEDA neurons regulate the secretion of PRL from the AL and α-MSH from

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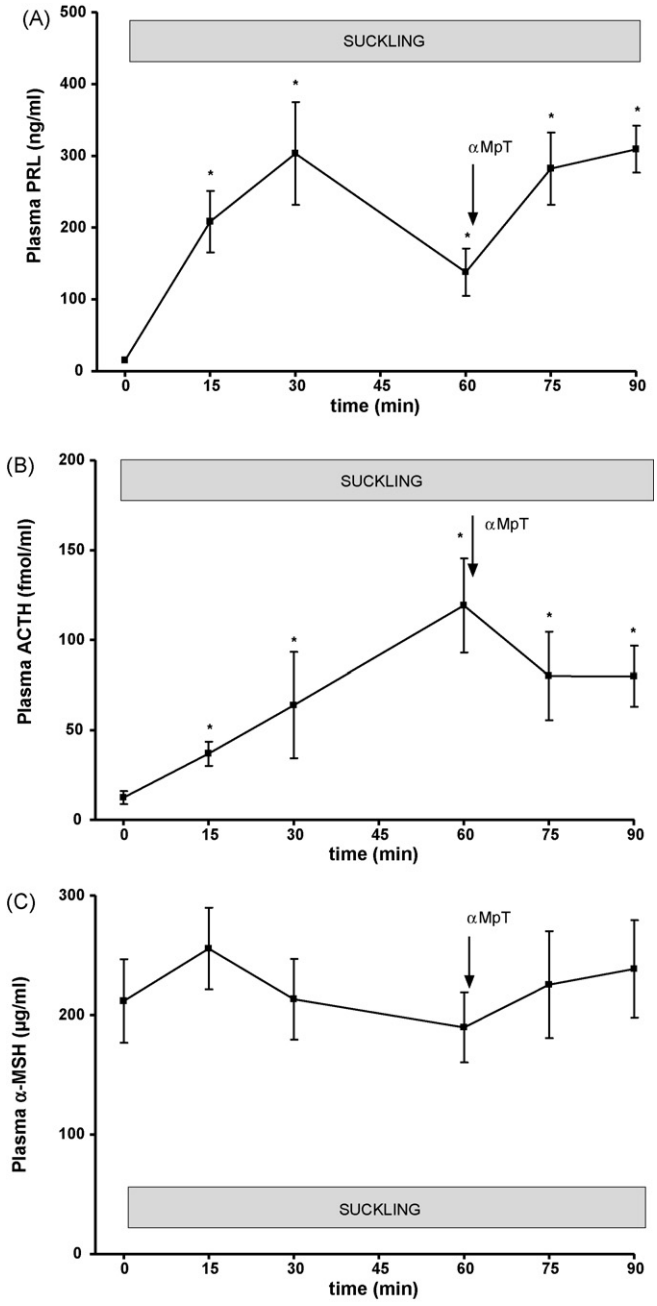

Fig. 1. Effect of suckling stimulus (at 0 min) and subsequent TH inhibition by α-MpT (8 mg/kg bw, iv) injection (at 60 min) on plasma PRL (A), ACTH (B) and α-MSH (C) levels. Each value represents the mean ± SEM (n = 6–8). Statistical significance compared to 0 time: $^{*}p < 0.05$.

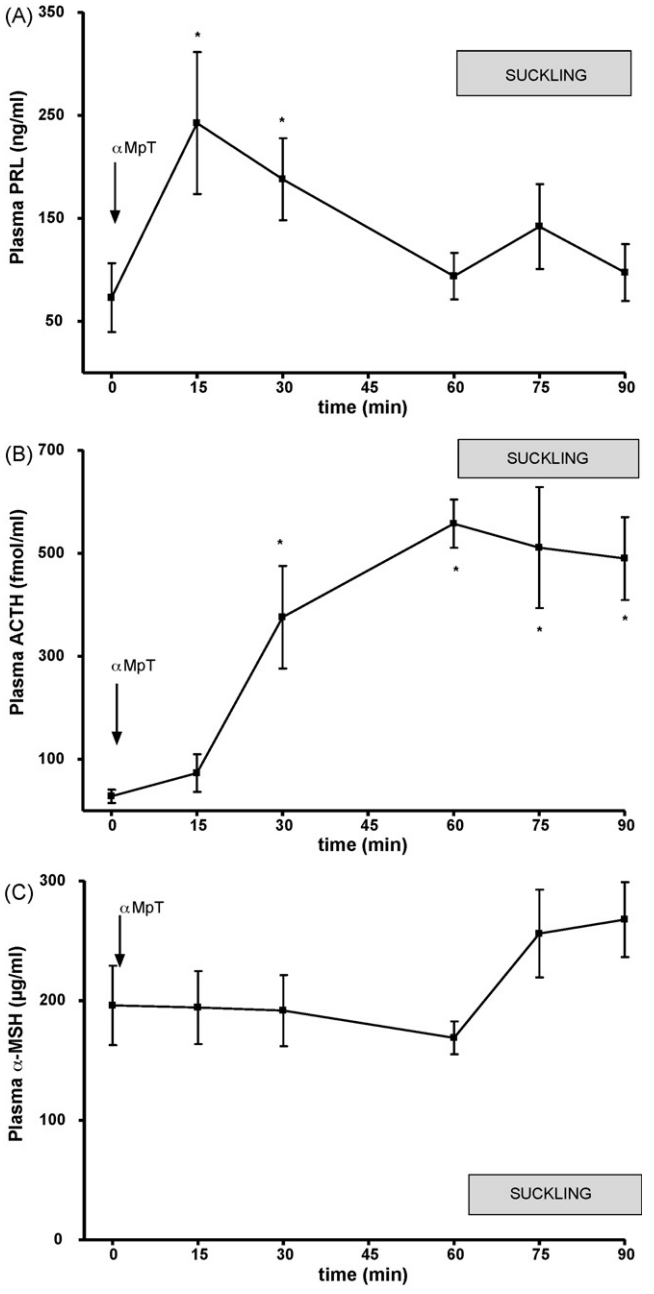

Fig. 2. Effect of TH inhibition by α-MpT (8 mg/kg bw, iv) injection (at 0 min) and subsequent suckling stimulus (at 60 min) on plasma PRL (A), ACTH (B) and α-MSH (C) levels. Each value represents the mean ± SEM (n = 6–8). Statistical significance compared to 0 time: $^{*}p < 0.05$.

the IL, although, at both terminal sites, DA inhibition is mediated through the same D2 type of DA receptors (D2R). During the last two decades, evidences revealed that DA released at the NL and the IL likely plays a role in the regulation of AL PRL secretion as well [7,14,22,27,29].

Regulation of adrenocorticotropic hormone (ACTH) and α-MSH, the two POMC derived peptides processed in the AL and the IL, respectively, are supposed to be different in lactating dams compared to either male or female rats [25]. For example, suckling, one of the strongest and best characterized stimuli for PRL release, induces an increase in plasma level of ACTH as well, without having any effect on α-MSH secretion [25,23]. At the same time, lactation associated with a reduced ACTH and PRL responses to various stressful stimuli [1,13,19,21,30]. Moreover, both ACTH and PRL secretions are sensitive to glucocorticoid feedback, whereas it has no effect on α-MSH release [2,5,15,16,20]. In addition, our recent findings have clearly shown that in lactating rats not the corticotrope releasing factor- (CRF) but NEDA neurons play a pivotal role in the increase of ACTH release induced by suckling stimulus [25]. At the same time DA has practically no influence on α-MSH secretion during lactation [25]. In agreement with this deletion of crucial players of the inhibitory regulation and/or processing of IL POMC, absence of D2R, prohormone convertase 2 (PC2), or its chaperone molecule 7B2, has also resulted in similar elevation of circulating ACTH [17,18,26].

The first and rate-limiting step of DA biosynthesis is the formation of L-3,4-dihydroxyphenylalanine (L-DOPA) from L-tyrosine. This is catalyzed by tyrosine hydroxylase (TH). TH activation by