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Knockdown of α_{2C}-adrenoceptors in the occipital cortex rescued long-term potentiation in hidden prenatally malnourished rats

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ABSTRACT

Moderate reduction in the protein content of the mother's diet calorically compensated by carbohydrates (the so-called "hidden" prenatal malnutrition) leads to increased neocortical expression of the α_{2C}-adrenoceptor subtype, together with decreased cortical release of noradrenaline and impaired long-term potentiation (LTP) and visuospatial memory performance during the rat postnatal life. In order to study whether overexpression of the α_{2C}-adrenoceptor subtype is causally related to the decreased indices of neocortical plasticity found in prenatally malnourished rats, we evaluated the effect of intracortical (occipital cortex) administration of an antisense oligodeoxynucleotide (ODN) raised against the α_{2C}-adrenoceptor mRNA on the LTP elicited *in vivo* in the occipital cortex of hidden prenatally malnourished rats. In addition, we compare the effect of the antisense ODN to that produced by systemical administration of the subtype-nonselective α_2-adrenoceptor antagonist atipamezole. Prenatal protein malnutrition led to impaired occipital cortex LTP together with increased expression of α_{2C}-adrenoceptors (about twice Bmax) in the same cortical region. [^{3}H]-rauwolscine binding assay showed that a 7-day intracortical antisense ODN treatment in the malnourished rats resulted in 50% knockdown of α_{2C}-adrenoceptor expression and, in addition, completely rescued the ability of the occipital cortex to develop and maintain long-term potentiation. Atipamezole (0.3 mg/kg i.p.) also led to full recovery of neocortical LTP in malnourished rats. The present results argue in favor of our original hypothesis that the deleterious effect of prenatal malnutrition on neocortical plasticity in the adult progeny is in part consequence of increased neocortical α_{2C}-adrenoceptor expression. This receptor subtype is known to be involved in the presynaptic control of noradrenaline release from central neurons, a neurotransmitter that critically influences LTP and memory formation.

1. Introduction

Moderate reduction of the protein content in the diet of pregnant rats, calorically compensated by carbohydrates, results in apparently normal development *in utero* of fetuses as assessed by normal maternal weight gain during pregnancy and normal body and brain weights of pups at birth (Resnick, Morgane, Hasson, & Miller, 1982). However, this insidious form of protein maternal malnutrition, the so-called "hidden" prenatal malnutrition (Resnick et al., 1982), results in altered noradrenergic function in the neocortex of the offspring, as revealed by increased concentration and release of cortical noradrenaline during early postnatal life, followed by decreased cortical release of the neurotransmitter during adulthood (Soto-Moyano et al., 1998a, 1998b). In addition, these animals showed increased neocortical expression of the α_{2C}-adrenoceptor subtype during postnatal life (Sierralta et al., 2006; Soto-Moyano et al., 2005). Together with the altered profile in central noradrenergic systems, the neocortex of adult prenatally malnourished animals shows weakened electrophysiological indices, including decreased ability of callosal–cortical synapses

Abbreviations: LTP, long-term potentiation; ODN, oligodeoxynucleotide; Bmax, maximum receptor density; K_D, dissociation constant.

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to perform temporal summation (Soto-Moyano et al., 1998a) and to maintain long-term synaptic potentiation (Hernández et al., 2008; Soto-Moyano et al., 2005).

It has been hypothesized that many of the deficits in functional plasticity observed in prenatally malnourished animals are due, at least in part, to the above mentioned modifications in brain noradrenergic systems (Soto-Moyano et al., 2005). In fact, central nervous system noradrenaline critically influences long-term potentiation (LTP) in cerebral cortex (Kobayashi, 2007; Komatsu, 1996; Marzo, Bai, & Otani, 2009; Nowicky, Christofi, & Bindman, 1992) and hippocampus (Bramham, Bacher-Svendsen, & Sarvey, 1997; Hopkins & Johnston, 1988; Marzo et al., 2009; O'Dell et al., 2010; Radisavljevic, Cepeda, Peacock, Buchwald, & Levine, 1994; Schimanski, Ali, Baker, & Nguyen, 2007; Swanson-Park et al., 1999) as well as memory formation (Crowe, Ng, & Gibbs, 1990; Gibbs, 1991; Sternberg, Korol, Novack, & McGaugh, 1986), through balanced activation of specific receptors. For instance, animal studies have revealed that β-adrenoceptor activation is associated with enhancement of LTP in the hippocampus (Bramham et al., 1997; Hopkins & Johnston, 1988; Radisavljevic et al., 1994; Schimanski et al., 2007) and memory facilitation (Crowe et al., 1990; Gibbs, 1991; Gibbs & Summers, 2000), while activation of α_2-adrenoceptors (Bunsey & Strupp, 1995; Devauges & Sara, 1990; Haapalinna, Sirviö, & Lammintausta, 1998; Haapalinna et al., 1999; Sara & Devauges, 1989), specially the α_{2C} subtype (Björklund et al., 1998, 1999, 2000), is related to decreased memory formation.

Since α_{2C}-adrenoceptors are involved in the presynaptic control of noradrenaline release from central neurons (Bücheler, Hadamek, & Hein, 2002; Kable, Murrin, & Bylund, 2000), the reported overexpression of the α_{2C}-adrenoceptor subtype found in prenatally malnourished rats (Soto-Moyano et al., 2005) may be well on the basis of the decreased indices of both cortical release of noradrenaline and neocortical plasticity (Soto-Moyano et al., 1998a, 2005) showing these animals at adulthood. However, caution must be exercised regarding this issue because these observations reveal correlational rather than causal relationships. Thus, the question of whether the deleterious effects of prenatal malnutrition on neocortical LTP and visuo-spatial memory in the adult progeny are consequence of the increased α_{2C}-adrenoceptor expression found in the neocortex of malnourished rats remains still open. The present study was designed to examine this question by studying the effect of intracortical (occipital cortex) administration of an antisense oligodeoxynucleotide (ODN) raised against the α_{2C}-adrenoceptor mRNA on the LTP elicited *in vivo* in the occipital cortex of hidden prenatally malnourished rats, and to compare the effect to that produced by systemical administration of the subtype-nonselective α_2-adrenoceptor antagonist atipamezole. The occipital cortex was chosen as a suitable region for the present study, since (i) there are more studies exploring the role of noradrenaline and adrenoceptors on occipital cortex LTP than in any other neocortical region, and (ii) a hundred of studies have been explored the occipital cortex as a target of prenatal undernutrition at morphological, electrophysiological and biochemical levels. In order to evaluate the effectiveness of the intracortical antisense ODN treatment to knockdown α_{2C}-adrenoceptor expression in malnourished animals, the α_{2C}-adrenoceptor density was determined by means of [^{3}H]-rauwolscine binding assay in membranes prepared from the occipital cortices.

2. Materials and methods

2.1. Animals and diets

The present investigation was performed following protocols approved by the Committee for the Ethical Use of Experimental Animals at INTA, University of Chile, in accordance to the NIH Guide for the Care and Use of Laboratory Animals (National Research Council., 1985). The experiments were carried out on male Sprague–Dawley rats born from mothers inbreeding at the INTA facilities, University of Chile, which were submitted to rearing procedures already described in the literature (Hernández et al., 2008). Briefly, female rats were fed isocaloric purified diets containing either normal (25% casein, providing 22.5% protein) or low (8% casein, providing 7.2% protein) amount of protein. The other components of the purified diets were as follows: (i) normal diet: carbohydrate, 50.2%; fat, 15.0%; vitamin mix, 1.0%; salt mix, 4.7%; water, 1.7%; cellulose, 4.2%; L-methionine, 0.4%. (ii) Low protein diet: carbohydrate, 66.5%; fat, 15.0%; vitamin mix, 1.0%; salt mix, 4.7%; water, 1.0%; cellulose, 4.2%; L-methionine, 0.4%. Both diets provided about 4.3 kcal/g. The dietary paradigm began 1 day after mating and continued throughout pregnancy. The body weight gain of pregnant mothers was controlled daily. At birth, all pups were weighed and litters were culled to eight male pups. Afterwards, pups born from mothers fed the 7.2% protein diet were fostered by well-nourished dams (22.5% protein diet) giving birth on that day. Pups born from mothers receiving the 22.5% protein diet were also fostered to well-nourished dams to equalize among groups other factors that may depend on the rearing conditions (i.e. stress due to cross-fostering). After weaning, at 22 days of age, all pups were fed a standard laboratory diet providing 22.5% protein. The body and brain weights of pups were determined at birth and at the age of experiments. Brain weight measurements were performed excluding cerebellum, brainstem and olfactory bulbs.

2.2. Intracortical antisense oligodeoxynucleotide (ODN) treatment

On day 53 of age, 21 previously malnourished animals were anesthetized with chloral hydrate (300 mg/kg i.p.), placed in a stereotaxic apparatus and their skulls exposed. A 1.5-mm diameter hole was drilled in the left occipital bone with center at the stereotaxic coordinates $A = 0.0$, $L = 3.5$ mm according to the atlas of Pellegrino and Cushman (1967), to approach the occipital cortex with a 30 gauge (0.25-mm OD) stainless steel hypodermic needle fixed to a micromanipulator. Once the hypodermic needle was placed into the occipital cortex (the beveled needle tip positioned to a depth of around 2 mm), it was secured to the skull with glass ionomere dental cement, severed 5 mm above the skull surface, and connected with a polyethylene tubing to a mini-osmotic pump delivering 0.5 μL/h (Alzet pump model 2002). The mini-osmotic pump was filled with 0.5 nmol/μL of a saline solution of either an antisense ODN ($n = 7$ rats) to knock-down α_{2C}-adrenoceptors or a mismatch ODN ($n = 7$ rats) with scrambled sequence of the same bases (Biosonda S.A., Santiago, Chile). Finally, the mini-osmotic pump was implanted subcutaneously on the back of each animal and the skin sutured. Similar stereotaxic surgery was made in control malnourished rats but employing mini-osmotic pumps filled with saline ($n = 7$ rats). The antisense ODN was directed against the 5′ end of the coding sequence of the α_{2C}-adrenoceptor, according to Fairbanks et al. (2002): 5′-CCA-TTC-GCC-CGC-GTC-GCT-CC-3′, and was phosphorothioate-modified at terminal nucleotides at the 5′ and 3′ ends to delay nuclease degradation. The mismatch version of the ODN was 5′-GCA-TGC-GCC-CTC-GTC-CCT-CC-3′, according to Fairbanks et al. (2002), being also phosphorothioate-modified at the two bases of each end.

In order to check knockdown of α_{2C}-adrenoceptor expression, on day 7 after pump implantation, once finished the electrophysiological experiment, tissue from the left occipital cortex was processed for α_{2C}-adrenoceptor binding assay as described by Soto-Moyano et al. (2005), while tissue arising from the right occipital cortex served as control.