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Research report

Phosphodiesterase-7 inhibition affects accumbal and hypothalamic thyrotropin-releasing hormone expression, feeding and anxiety behavior of rats

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HIGHLIGHTS

- Accumbal administration of a PDE7 inhibitor induces anxiolysis in rats.
- Peripheral administration of PDE7 inhibitor is anxiolytic or anorexigenic.
- Behavioral effects of PDE7 inhibition could be mediated by NAcc and PVN TRH.

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ABSTRACT

Thyrotropin-releasing hormone (TRH) has anorexigenic and anxiolytic functions when injected intraventricularly. Nucleus accumbens (NAcc) is a possible brain region involved, since it expresses proTRH. TRH from hypothalamic paraventricular nucleus (PVN) has a food intake-regulating role. TRHergic pathways of NAcc and PVN are implicated in anxiety and feeding. Both behaviors depend on cAMP and phosphorylated-cAMP response element binding protein (pCREB) intracellular levels.

Intracellular levels of cAMP are controlled by the degrading activity of phosphodiesterases (PDEs). Since TRH transcription is activated by pCREB, a specific inhibitor of PDE7B may regulate TRH-induced effects on anxiety and feeding.

We evaluated the effectiveness of an intra-accumbal and intraperitoneal (i.p.) administration of a PDE7 inhibitor (BRL-50481) on rats' anxiety-like behavior and food intake; also on TRH mRNA and protein expression in NAcc and PVN to define its mediating role on the PDE7 inhibitor-induced behavioral changes.

Accumbal injection of 4 μg/0.3 μL of PDE7 inhibitor decreased rats' anxiety. The i.p. injection of 0.2 mg/kg of the inhibitor was able to increase the PVN TRH mRNA expression and to decrease feeding but did not change animals' anxiety levels; in contrast, 2 mg/kg b.w inhibitor enhanced accumbal TRH mRNA, induced anxiolysis with no change in food intake.

PDE7 inhibitor induced anxiolytic and anorexigenic like behavior depending on the dose used. Results supported hypothalamic TRH mediated feeding-reduction effects, and accumbal TRH mediation of inhibitor-induced anxiolysis. Thus, an i.p dose of this inhibitor might be reducing anxiety with no change in feeding, which could be useful for obese patients.

1. Introduction

Anxiety and feeding disorders are highly prevalent co morbidities: about 50% of anorexic and over 20% of obese/hyperphagic patients, present phobias or different anxious behaviors [1–4].

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Some medication for treating mood disorders improves anxiety symptoms but at the same time increases food intake and body weight in about 32% of the users [5]; i.e. anxiolytic pharmaceuticals that target GABAergic systems, such as benzodiazepines, the most common medication for anxiety disorders, also increase feeding [6–8], which is undesirable for patients with obesity.

Mood and feeding disorders have similar neural substrates, for example amygdala, hypothalamus, ventral tegmental area (VTA) and NAcc are known to participate in the regulation of those behaviors [9–14]. NAcc involvement in humans' mood disorders results from the anxiolysis they show when NAcc is the target of deep brain stimulation (DBS) [15,16], or by ablation of the region [17]. NAcc implication in feeding has been defined by studying patients with intractable anorexia, who also show improved appetite and feeding after DBS of that nucleus [18].

Interestingly, TRH is widely distributed in the brain and it has been implicated with anorexigenic and anxiolytic effects after its central or peripheral administration [19–22]. Expressed TRH in NAcc and TRH synthesizing neurons of the hypothalamic paraventricular nucleus (PVN) seem to participate in the regulation of animals' feeding behavior [23,24].

For example, the direct injection of TRH in NAcc reduces food consumption and feeding motivation in rodents [21,25], while TRH's involvement in anxiety is supported by reduced burying behavior in the defensive burying test (DBT) [20], by the lower acoustic startle and freezing in fear conditioning tests [26] and by the attenuated punishment behavior in conflict tests that animals show after i.c.v. or peripheral TRH injection [27]. However, the participation of the accumbal TRHergic system in anxiety has not been demonstrated.

TRH gene contains a cAMP response element (CRE) in its promoter region [28], where phosphorylated CREB (pCREB) binds and stimulates peptide gene transcription [29]. Such regulation of TRH expression by pCREB may occur in NAcc and in other brain regions.

Regionally, PVN TRH expression may be reduced by the active thyroid hormone (TH), triyodotyronine (T_3). Thus, locally, pCREB effects on PVN TRH transcription may be overcome by the pCREB-induced increased resultant activity of deiodinase 2 (DIO2), a selenoenzyme that catalyzes thyroxine (T_4) deiodination to T_3 [24].

Intracellular cAMP concentration is in part regulated by the activity of phosphodiesterases (PDEs). Interest in PDE inhibitors has recently grown given that these chemicals enhance cAMP content and influence behaviors' display by decreasing its degradation. The 11 isoforms of PDEs are differentially expressed in brain regions and their activities are finely and specifically regulated, in such a way that diverse CREB activated processes and behaviors may be controlled depending on the used inhibitor and on the injected brain region [30–34]. For example, higher CREB expression and its phosphorylation in amygdala and in NAcc are associated with ethanol induced anxiolytic effects [35–39]. Also, increasing accumbal CREB activity inhibits anxiety responses of rodents to light and to isolation, while a dominant negative mutant rodent strain with decreased CREB activity in the same region, presents the opposite effect [40,41].

PDE7B is one of the two isoforms of PDE7 (A and B) and also one of the most abundant PDEs expressed in NAcc, as well as, in PVN [42]. PDE7 is specifically inhibited by the compound 3 (N,N dimethylsulfonamido) 4 methyl nitrobenzene (BRL 50481). Given that only the B isoform is expressed in NAcc and in PVN, changes in genetic expression in those regions may only be attributable to PDE7B inhibition.

In this study, we first evaluated the ability of this specific PDE7 inhibitor to behave as an anxiolytic and anorexigenic factor when injected i.p. to Wistar rats; and second, its suitableness for reducing anxiety without stimulating food intake. In addition we studied the effects of an accumbal injection of the PDE7 inhibitor on feeding and anxiety behaviors of rats; furthermore we analyzed changes in the expression of TRH, CREB and pCREB in PVN and NAcc after the i.p. administration of the inhibitor.

Results supported potentially beneficial effects of the peripheral administration of this inhibitor on both feeding and anxiety behaviors.

2. Materials and methods

2.1. Animals

Wistar male rats (270–300 g) were housed individually and maintained in a 12 h light/dark cycle (0700–1900 h), temperature (23 ± 1 °C) with *ad libitum* access to chow pellets (Lab Rodent Diet 5001; PMI Feeds, Brentwood, MO, USA) and tap water, for one week until the experiments started. Diets and treatments were approved by the Ethics Committee and the Project Commission of the National Institute of Psychiatry Ramón de la Fuente Muñiz, in agreement with the National Institutes of Health Guide (NIH Publications No 8023).

2.1.1. Cannulation and microinjection procedures

Animals were anaesthetized with an i.p. administration of ketamine 100 mg/kg of body weight (b.w.) (Anesket, Pisa Agropecuaria, Hidalgo, México) and xylazine 13 mg/kg b.w. (Procin, Pisa Agropecuaria, Hidalgo, México) and placed in a stereotaxic apparatus. A stainless steel guide cannula (23 gauge) was implanted bilaterally in the NAcc shell (n = 7/group) (AP 1.7, L ± 1 to bregma, DV −6.8) [43]. After 4 days of recovery, animals were administered with either vehicle (0.3 μL of saline [0.9% NaCl, Sigma Aldrich, St. Louis, MO, USA]) or PDE7 inhibitor (BRL 50481, Tocris, Bristol, UK) (4 μg/0.3 μL). To perform the injection, an injector (a 28 gauge for micro injection) attached to a 5 μL Hamilton syringe was inserted into the cannula (n = 4/group). After 24 h, rats were subjected to the DB anxiety test. Thirty minutes after performing the behavioral test, animals were decapitated, their brains excised and NAcc dissected to analyze TRH expression by RT PCR. In this experiment, the inhibitor dose was based on study by Smith et al., [44] who described the kinetic characteristics of the compound; K_i for BRL 50481 *in vitro* is 180 ± 10 nM.

In a different trial, we cannulated 18 animals in the left NAcc shell and after 4 days of recovery we formed 3 groups: 1) veh-veh, who received one daily injection of vehicle (2.5% dimethyl sulfoxide [DMSO, Sigma Aldrich, St. Louis, MO, USA] in saline [0.9% NaCl, Sigma Aldrich, St. Louis, MO, USA]) for three days at 0900 and on the third day at 1300 h an additional administration of vehicle; 2) inverted oligonucleotide iODN-I group, received a daily injection of 10 μg/0.3 μL of TRH iODN and on the third day at 1300, a 4 μg/0.3 μL dose of PDE7 inhibitor; 3) antisense asODN-I group, received for three days 10 μg/0.3 μL of TRH asODN and on the third day at 1300 h, an injection of the same dose of PDE7 inhibitor as group '2′. At 1400 h, all animals were subjected to the elevated plus maze (EPM) for 10 min; video recordings of the test were later analyzed for anxiety parameters (described below). ODNs of rat prepro-TRH gene were synthesized in the Biotechnology Institute (National University of México) facilities. ODNs were phosphothioated to be resistant to nucleases and were the same used by Landa et al., 2007 [45]. Antisense ODN asODN: 5′-AAC CAA GGT CCC GGC ATC CTG GA-3′, and an inverted iODN: 5′-AGG TCC TAC GGC CCT GGA ACC AA-3′. We Blast tested the specificity of the asODNs sequence used and did not find homology with any other mRNA.

2.1.2. Anxiety levels after an i.p administration of PDE7 inhibitor

Given that to our knowledge this inhibitor had not been tested for anxiolytic properties, we used an i.p dose of 2 mg/kg, which was employed by Nandhakumar et al. [46] in an epilepsy model and a