

The Relevance of Animal Models of Anorexia: Link with Depression and Anxiety

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Published Date: July 18, 2016

ABSTRACT

Anorexia nervosa (**AN**) is a complex and multifactorial illness which represents an important public health burden. Patients suffering from AN have high comorbidity with other psychiatric disorders, such as depression and anxiety, which complicate the treatment and delays patient recovery. There is a crucial need to better understand the underlying mechanisms involved in the pathophysiology of AN in order to develop novel pharmacological strategies accompanying the valuable psychological therapy. Animal models, specifically the ones using rodents, have been widely used in biomedical research given their similarities in anatomy, physiology and genetics, allowing inferences to be drawn about human biology. In this review, we focus on the description of the most commonly used animal models of anorexia, which share some traits with AN patients.

First, we discuss the genetic model *anx/anx*, and then we address activity-based anorexia, social defeat stress and dehydration-induced anorexia paradigms. Overall, animals in these models have mood disturbances, brain and peripheral alterations that are similar to those observed in anorexic patients; therefore, these rodent paradigms are helpful to identify brain neuropeptides and neurotransmitters' systems involved in the display of those alterations. Finally, we discuss some recent findings using the dehydration-induced anorexia model and administration of phosphodiesterases inhibitors as potential anxiolytic and antidepressant agents. In addition, we show results related to the mediation of central thyrotropin-releasing hormone as an indirect neuropeptide target of those inhibitors' effects. Despite the limitations of the animal models described in this review, their advantages are numerous and thus, are still valuable to gain insight in the pathophysiology of eating disorders.

Keywords: Anorexia nervosa; Animal models; Depression; Anxiety

Abbreviations: **5-HT:** Serotonin; **ABA:** Activity-based anorexia; **ACC:** Acetyl-CoA carboxylase; **ACTH:** Adrenocorticotrophic hormone; **AgRP:** Agouti-related protein; **AMPK:** AMP-activated protein kinase; **AN:** Anorexia nervosa; **aPVN:** Anterior subdivision of the hypothalamic paraventricular nucleus; **ARC:** Hypothalamic arcuate nucleus; **BDNF:** Brain-derived neurotrophic factor; **BRL-50481:** N-dimethylsulfonamido-4- methyl-nitrobenzene; **B.W:** Body weight; **cAMP:** Cyclic adenosine monophosphate; **CART:** Cocaine and amphetamine-regulated transcript; **CRH:** corticotropin-releasing hormone; **CSF:** Cerebrospinal fluid; **DA:** Dopamine; **DBT:** Defensive-burying test; **DIA:** Dehydration-induced anorexia; **FST:** Forced-swim test; **HPA:** Hypothalamic-pituitary-adrenal axis; **HPT:** Hypothalamic-pituitary-thyroid axis; **i.c.v.:** Intracerebroventricular; **i.p.:** Intraperitoneal; **IR:** immunoreactive; **LC:** Locus coeruleus; **LHA:** Lateral hypothalamic area; **MCH:** melanin-concentrating hormone; **mpVN:** Medial subdivision of the hypothalamic paraventricular nucleus; **NAcc:** Nucleus accumbens; **NE:** Norepinephrine; **NPY:** Neuropeptide Y; **pCREB:** phosphorylated form of the cAMP-response element binding protein; **PDE:** Phosphodiesterase; **PKA:** Protein kinase A; **POMC:** Proopiomelanocortin; **PVN:** Paraventricular hypothalamic nucleus; **RT-PCR:** reverse transcription-polymerase chain reaction; **SDS:** Social defeat stress; **T₃:** triiodothyronine; **TNF:** Tumoral necrosis factor; **TRH:** Thyrotropin-releasing hormone; **TSH:** Thyrotropin.

INTRODUCTION

Anorexia nervosa (**AN**) is a psychiatric disorder with physical, cognitive and socio-emotional abnormalities [1]. Diagnosis criteria encompass restriction of food intake relative to energy demands, anxiety, obsessive traits, distorted body image and pathological thoughts about eating, weight, and body shape [2]. This disorder presents two patterns: individuals with the extreme weight loss caused by dietary restriction or those with binge eating followed by purge periods [2]. Furthermore, up to 80% of anorexic patients report that excessive exercise is involved in their pathological state [3,4]. Psychiatric comorbidity in AN patients is high in relation to anxiety

and depression [5,6]; medical complications and lifetime mortality rates are amongst the highest in comparison with other psychiatric disorders [7]. However, due to obvious ethical reasons, its complex aetiology and because of the psychological component of the disease, the use of animal models assessing AN aspects has become relevant [8,9].

There are biological, psychological and/or environmental factors that cause AN; the analysis of neurophysiological and neurobiological changes associated with core symptoms of AN, helps to understand its underlying mechanisms and to find effective treatments for the disorder. Several useful paradigms have been developed to study specific traits of human AN. This review will focus on a genetic model and three environmental-induced anorexia models developed in rodents. Moreover, we will discuss some recent findings of our laboratory using one of these models in relation to depression and anxiety.

Anx/Anx Mice

In 1976, *anx/anx* mutant mice appeared spontaneously in Jackson Laboratories (Bar Harbor, USA). The phenotype of these animals is characterized by a decrease in food intake and in B.W. detectable at 9 days of age, emaciated appearance and premature death at 3-5 weeks of age. Moreover, *anx/anx* animals display abnormal behaviors such as, head weaving, body tremors, hyperactivity and uncoordinated gait [10]. *Anx/anx* phenotype results from a recessive mutation in chromosome 2 and it is associated with a down-regulated *Ndufa1* gene expression, which encodes for a protein of mitochondrial complex 1. *Anx/anx* mutation also alters the expression of several genes involved in feeding behavior, locomotion and reward. *Anx/anx* animals show alterations in hypothalamic peptidergic systems, such as orexigenic neuropeptide Y/agouti-related protein (**NPY/AgRP**) and anorexigenic cocaine and amphetamine-regulated transcript/proopiomelanocortin (**CART/POMC**) systems. Moreover, there is a decreased NPY- and AgRP-immunoreactive (**IR**) positive terminals and increased IR signal in perikarya of hypothalamic arcuate nucleus (**ARC**) neurons, suggesting cell body accumulation [11] may be related to the anorexia of these animals. This effect is linked to the ARC serotonergic hyper-innervations [12] and to those in hippocampus, cortex, olfactory bulb and cerebellum [13]. *Anx/anx* animals present reduced expression of NPY receptors, Y1R and Y5R, along with low POMC and CART mRNA's expression [14,15], possibly as counter-regulatory mechanisms against starvation. The decreased POMC/CART mRNA and IR signal could also account for a neurodegenerative process, since leptin serum levels are decreased and this hormone promotes the outgrowth of ARC projections from postnatal day 7 [16]. Dopamine (**DA**) and its metabolites (homovanillic acid and 3-methoxytyramine) levels are diminished in the striatum of *anx/anx* animals, which may contribute to animals' eating and motor disturbances [15,17]. An altered norepinephrine (**NE**) neurotransmission may also be implicated in *anx/anx* phenotype, since expressions of NE degrading enzyme monoamine oxidase A and the NE transporters in the locus coeruleus (**LC**) of those animals, decrease [12]. NE system's change may be an adaptive response to starvation, since NE injections into hypothalamic paraventricular nucleus (**PVN**) stimulate food intake [18].

Interestingly, the majority of NE inputs to PVN proceed from the LC [19], where alterations of the neurotransmitter's system are described. Several genes involved in immune/inflammatory response (molecules of the major histocompatibility complex class I and II, complement factors that indicate activation of glia and macrophages, pro-inflammatory cytokines) are up-regulated [20,21]. TNF (tumoral necrosis factor), interleukin-1, interleukin-6 and interferon- γ are pro-inflammatory cytokines known to inhibit food intake, whose synthesis is increased by some starvation-induced peripheral metabolites, such as high free fatty acids. This indirectly leads to glucose intolerance [22], due to the stimulation of macrophage infiltration and inflammation of pancreatic β -cells, and to the associated impaired insulin effects. Evidence regarding immunological response suggests that anorexia phenotype in *anx/anx* animals may be associated to an autoimmune process, suggested by the antibodies directed against several hypothalamic peptides that anorexic patients show [17,23]. Regarding the associated comorbidities (depression and anxiety) to anorexia, there are no reports studying behavioral alterations in these animals, despite of their associated DA and serotonin (**5-HT**) systems dysfunction. All *anx/anx* data make it a natural useful genetic approach to explore the physiological alterations displayed by AN patients.

Activity-based Anorexia (ABA)

ABA paradigm is also known as self-starvation/semi-starvation-induced hyperactivity/food restriction-induced hyperactivity or activity anorexia and was introduced in 1967 using running wheels for modelling rats' exercise [24]. This model reproduces hyperactive behaviors characteristic of AN, reduced food intake in hungry rodents, weight loss and desire for activity along with physiological consequences of starvation [25]. A rewarding effect of running on a wheel is suggested as rodents are motivated to perform diverse operant responses to gain access to it [26]. When *ad libitum* fed male rodents have free access to voluntary exercise on a running wheel, they experience a transient hypophagia in comparison to controls (rats without running wheel access) [27]. Rodents that are maintained on a severely restricted feeding schedule (usually 1 h per day of food access) and with access to a running wheel, show excessive exercise leading to a decrease in B.W. Eventually, energy expenditure by wheel running exceeds caloric intake and animals may starve themselves to death. ABA rats have metabolic, endocrine and neural abnormalities that mimic those seen in the human disorder, such as increased hypothalamic NE, DA, and 5-HT neurotransmission. Tyrosine administration reverses high 5-HT levels induced by ABA and restores food consumption, improves cognitive behavior and activity performance [28]. Dopaminergic antagonist (cis-flupenthixol) reduces activity, increases B.W. and food intake [29]. Taken together, those evidences suggest an interaction between DAergic and 5-HTergic systems in the regulation of feeding in this paradigm. Interestingly, leptin suppresses ABA behavior [30,31], which suggests its interaction with the DA system. Controversially, a central leptin injection leads to lower running wheel activity but is not always associated with decreased food consumption [32,33]. Up-regulation of AgRP and NPY mRNA expression, and down-regulation

of POMC and CART expression in ARC are observed in ABA, when compared to control rats [31,34,35], which points out that ARC neuropeptidergic systems involved in feeding regulation respond accordingly to starvation in these animals. In contrast, ABA animals do not have increased melanin-concentrating hormone (**MCH**) and orexin expression in lateral hypothalamic area (**LHA**) or reduce corticotropin-releasing hormone (**CRH**) expression in PVN [31], contributing to their anorexic behavior. Regarding mood alterations in this model, when C57BL/6J mice with low anxiety levels are subjected to ABA, they display reduced wheel running; while DBA/2J mice with high anxiety levels show high activity-related behavior [36].

Social Defeat Stress (SDS)

SDS arises at first as a paradigm of anxiety and depression [37]. In this model, experimenter places an intruder rodent in the home cage of another rodent (resident), leading to aggressive behaviors between the two animals and promotes a dominant and subordinate situation. SDS causes a decrease in B.W. following 1 hour of the stress session [38], and up to 5 weeks, which correlates with a decreased food intake [39]. Hypothalamic malonyl-CoA concentration increases in SDS rats by the inhibition of AMP-activated protein kinase (**AMPK**) and the activation of the acetyl-CoA carboxylase (**ACC**) [39]; increased malonyl-CoA inhibits ARC NPY/AgRP and enhances POMC/CART expression [40-42], which may contribute to the anorexia of these animals. Additionally, in SDS rodents, expression of DA receptor type 1 decreases in the prefrontal cortex and amygdala [43], whereas DA content increases in ventral tegmental area [44], which suggests activation of the DA system that is related to reduced food intake [45,46]; besides, it is noteworthy that a link between function of dopaminergic system and depression has been established [47-49]. These animals also present hypothalamic-pituitary-adrenal (**HPA**) axis hyperactivation evinced by increased serum adrenocorticotrophic hormone (**ACTH**) and corticosterone levels [50,51]. Centrally, animals show diminished expression but increased translation of PVN CRH mRNA along with higher pituitary CRH-R1 receptor internalization in response to CRH content enhancement [51]; those changes are associated with depression. Interestingly, depressive patients show elevated CRH cerebrospinal fluid (**CSF**) levels and deregulation of HPA axis [52], whereas the antidepressants treatment normalizes CSF CRH content [53,54]. Serotonergic neurotransmission is affected in SDS; density of hippocampal 5-HT transporters decreases and pretreatment of SDS animals with a selective serotonin reuptake inhibitor (fluoxetine) reduces their hypophagia, B.W. loss and anxiety-like behavior [55]. Furthermore, SDS rats show decreased leptin mRNA expression in white adipose tissue, along with reduced leptin and cholesterol serum levels [56], disturbances presented by anorexic and depressive patients [57-60], and related to reduced serotonin central levels [61,62]. Although the decrease in leptin serum content seems to be the normal adaptive response to cope with negative energy balance, it has been proposed that SDS animals present an altered leptin signaling pathway, since they show a decrease in hypothalamic AMPK content, which increases malonyl-CoA leading to inhibition of NPY/AgRP and enhancement of POMC/CART expression [56]. SDS animals display behavioral alterations

such as anxiety- and depression-like behaviors evinced by increased immobility in the forced swim test (**FST**) [63], decreased locomotor activity and social interaction with a non-aggressive rodent [38,50], higher time spent in the dark box of the light/dark preference test [64], and an altered pattern of grooming behavior [65]. This animal model accounts for physiological and behavioral alterations seen in AN patients, but one of its limitations is the uncertain identification of the cause from the effect, since anorexia could be a symptom of depression, or depression and/or anxiety could be secondary to anorexia.

Dehydration-induced Anorexia (DIA)

AN patients present osmoregulation impairment and renal complications due to their fluid intake [66-68]. Watts and colleagues developed DIA model [69], which consists in offering rats a hypertonic solution of NaCl (2.5%) replacing tap water during 4 to 14 days [69,70]. DIA involves a physiological adaptation that limits the intake of osmolytes from food, diminishes the amount of water used during digestion and thus, helps maintaining the integrity of fluid compartments [71]. Animals subjected to DIA have reduced food intake associated with a negative energy balance, when compared to control animals that drink tap water [69,70,72]. After five days of drinking saline solution, DIA rodents reduce their B.W. to 69% and become anorexic despite their negative energy balance, which is evinced by the increased corticosterone and decreased leptin, and triiodothyronine (**T₃**) serum levels [72-74]. Centrally, DIA causes an up-regulation of NPY and down-regulation of POMC mRNAs in the ARC, CRH-R2 mRNA in the PVN [70,72,75]. Overall these findings show that the ARC is responding to the negative energy balance of DIA rats, but that alterations in LHA or PVN peptides system seem the ones involved on aberrant anorexic behavior of DIA. Even more CRH-R2 antagonist administration into the PVN attenuates anorexia in these animals [70]. Rats subjected to DIA have increased thyrotropin-releasing hormone (**TRH**) mRNA levels in anterior (**aPVN**) and caudal PVN subdivisions, while in medial PVN (**mPVN**), decreases [74]. Furthermore, anorexia displayed by DIA rats may be due in part to the activated TRHergic pathway in aPVN, since its projections reach brain areas related to food intake control [76]. Such aPVN TRH mRNA enhancement seems caused by high activities of type 2 deiodinase and pyroglutamyl-aminopeptidase II enzymes [74], as well as by lower thyroid hormone transporter, monocarboxylate transporter 8 expression in aPVN in comparison to controls [77]. DIA is a well-established and useful model to study neuronal circuits and involved neuropeptides in feeding behavior, having the advantage that anorexia can be quickly reversed when tap water is available [69]. DIA-induced changes in brain and periphery highlight the varied and complex interactions of several neural systems that allow animals to respond to environmental pressures by displaying adaptive behaviors.

MOOD DISORDERS AND ANOREXIA

Mood disorders such as anxiety and depression are known to affect feeding and to lead individuals to eat a greater or lesser amount of food than the needed to cover their energy demands.

This is also true for rodents making animal models valuable to study the interactions between those behaviors. The specific anxiety-induced feeding response depends on different factors, such as genetics, age and gender of the individual, as well as environmental stress situations.

Stress generally underlies both mood disorders. Modulation of neuroendocrine and monoaminergic pathways in specific brain areas involved in stressful situations may promote depressive and anxiety-like behaviors in rats, causing a decreased consumption only of palatable food [78]. Thus, an interesting approach to regulate anxiety or depression along with modification of food intake in anorexic patients is the identification of dual or single effects of neuropeptides' pathways in mood and feeding disorders as well as, to design adequate new pharmaceutical therapies to treat these pathologies.

Anorexia and Anxiety

Anorexia and anxiety are comorbidities found in 20 % of the cases [79,80], mainly because they share activation of same neural substrates and pathways. An example is nucleus accumbens (**NAcc**), the ventral part of the caudate-putamen that has been involved in regulation of anxiety and feeding behaviors [81-83].

In fact, accumbal deep-brain stimulation is used to reduce anxiety in humans and to improve appetite when pharmacological therapies do not succeed [84]. Hedonic and motivational aspects of food intake can also be regulated by NAcc [85]. An accumbal's peptidergic pathway involved in both mood disorders and feeding is TRH, since its intra-accumbal injection decreases feeding in energy-restricted animals [86] overcoming the activation of orexigenic peptides' expression that is induced by their negative energy balance [87]. In our laboratory, we find that the direct administration of TRH in NAcc also decreases rats' feeding motivation, when subjected to the progressive-ratio operant-conditioning test [85].

TRH participation in anxiety is evinced by the observed effect in intracerebroventricular (**i.c.v.**)-injected animals that reduce their burying behavior when subjected to the defensive-burying test (**DBT**) [88], and by their decreased acoustic startle and freezing response during fear conditioning tests [89]; also by the attenuation of rats' punishment behavior after a peripheral administration of TRH, when performing a conflict test [90].

By analyzing TRH expression and content in limbic brain areas of animals performing the DBT, the amygdalar neuropeptide's pathway seems involved in anxiety by the inhibited TRHergic neurons that animals show along with their facilitated anxiety-like behavior [88].

However, brain regions involved in TRH-induced changes in anxiety behavior of rats that present food-avoiding behavior are not identified yet. Thus, we analyzed the accumbal TRH expression changes in anorexic rats that were subjected to 5 days of dehydration stress (DIA model described above) [69,72].

Rats' B.W. decreased to 79% to that of controls (=100%) along with a dramatic reduction in food intake to 75% by the 5th day of DIA paradigm, evincing their aberrant anorexic behavior [69,73,74] (Figure 1,2).

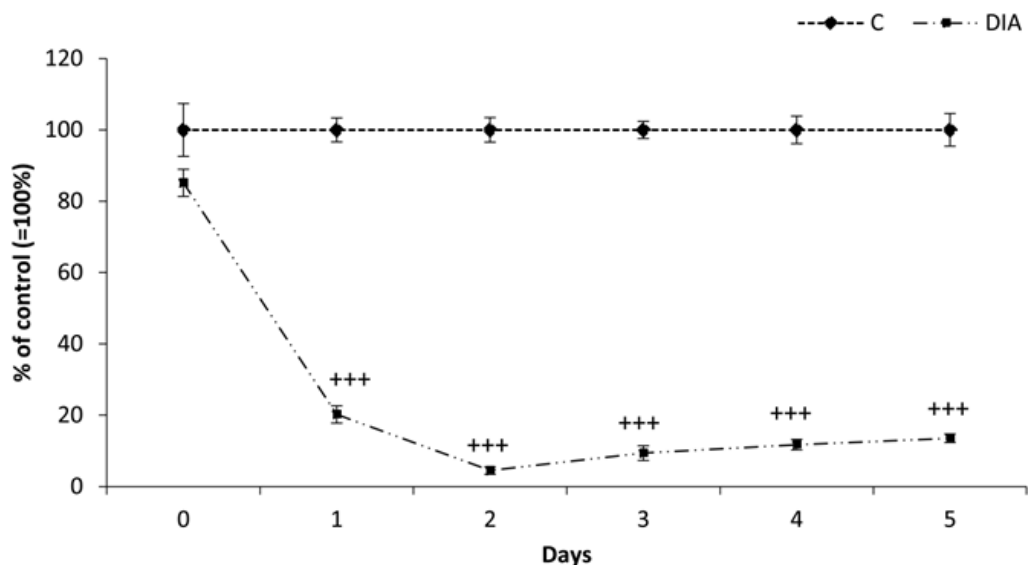


Figure 1: Food intake of control and DIA animals during 5 days of experiment. DIA group is offered as drinking water, a 2.5 % NaCl solution with food offered *ad libitum*, whereas controls drink tap water. Values are the mean $\pm$ SEM of food intake registered daily, and are expressed as % of control data (=100%) (n=8/group). Two-way ANOVA of repeated measures, followed by Holm-Sidak post-hoc test showed significant differences when $^{+++}p<0.001$ vs controls each day. **C:** control, **DIA:** dehydration-induced anorexia.

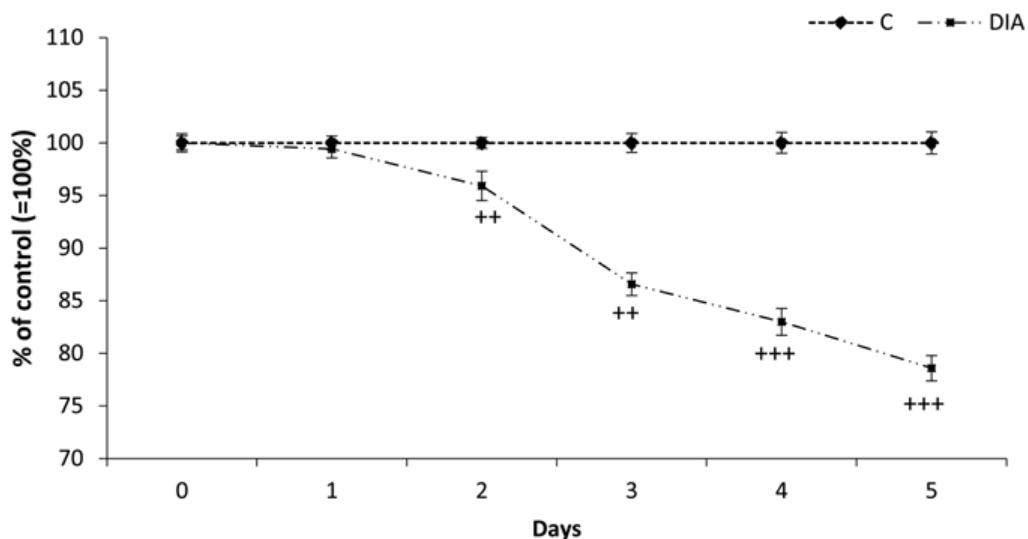


Figure 2: Body weight of control and DIA animals during 5 days of the experiment. DIA group is offered as drinking water, a 2.5 % NaCl solution with food offered *ad libitum*, whereas controls drink tap water. Values are the mean $\pm$ SEM of body weight registered daily, and are expressed as % of control data (=100%) (n=8/group). Two-way ANOVA of repeated measures, followed by Holm-Sidak post-hoc test showed significant differences when $^{**}p<0.01$, $^{+++}p<0.001$ vs controls each day. **C:** control, **DIA:** dehydration-induced anorexia.

Interestingly, DIA male adult rats showed 4 times higher scores of anxiety-like behavior when performing the DBT than controls on the 5th day of the experiment. Accordingly, we have previously observed a similar increment in time spent burying the electric prod of dehydrated-anorexic females [72]. Given that animals subjected to DIA presented not only food-avoiding behavior but also high anxiety levels, we tried to associate TRH system participation also in the mood disorder.

Accumbal TRH mRNA levels of DIA increased when compared to euhydrated male rats sacrificed on the 5th day of dehydration (Figure 3). TRH transcription may be activated by the phosphorylated form of the cAMP-response element binding protein (**pCREB**) as a result of increased intracellular cyclic adenosine monophosphate (**cAMP**) concentration [91], and which returns to basal levels by phosphodiesterase enzymes (PDEs, enzymes responsible for breaking down cAMP) activities [92]. Inhibition of specific PDEs augments pCREB protein levels that are associated with ethanol and isolation-induced anxiolysis [93-96]. Besides, CREB-negative mutants present higher anxious behaviors than wild-type mice [97].

Interestingly, mRNAs of TRH [98] and a specific PDE, the PDE7B, are expressed in NAcc [99]. Therefore, we analyzed the changes in TRH expression by injecting i.p. a drug that inhibits PDE7B, the N-dimethylsulfonamido-4-methyl-nitrobenzene (**BRL-50481**) [100] (Figure 3). A second isoform of PDE7 family is the 7A, but as it is not expressed in NAcc, the administration of the inhibitor will only act on the 7B type enzyme. Thus, we evaluated its potential anxiolytic effect on anorexic rats, and TRH system mediation.

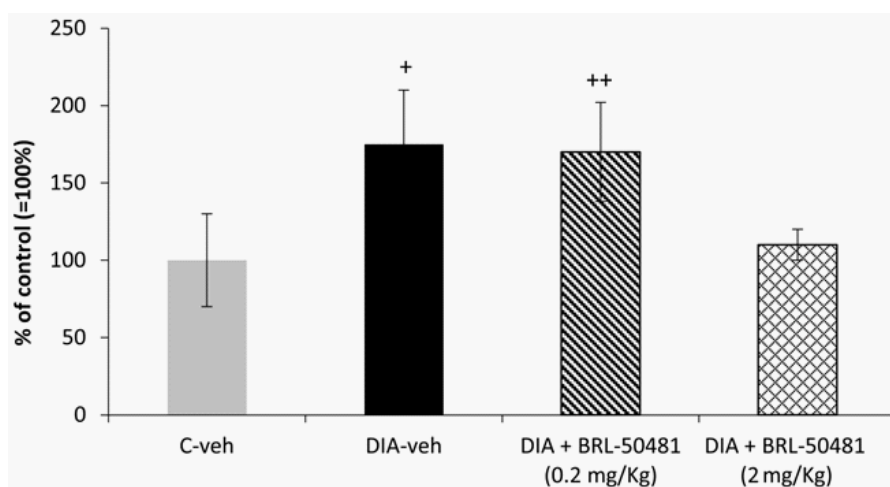


Figure 3: TRH mRNA content in nucleus accumbens of control and DIA rats before and after being injected i.p. with vehicle (**veh**) or two doses (0.2 or 2 mg/Kg of B.W.) of PDE7B inhibitor (BRL-50481), and sacrificed 30 min after performing the DBT. Data are the mean $\pm$ SEM of arbitrary units of the ratio cDNA of TRH/cDNA of cyclophilin evaluated by **RT-PCR** and expressed as percentage of control values (=100%). One-way ANOVA, followed by a Fisher's post-hoc test showed significant differences when * $p < 0.05$, ** $p < 0.01$ vs. C-veh. **C:** control, **DIA:** dehydration-induced anorexia.

Results supported that anorexic behavior in DIA rats could be related to the increased accumbal TRHergic pathway function. Regarding anxiety, the long time spent burying the prod of DIA rats along with activated accumbal expression of TRH was unexpected since the peptide is implicated with anxiolytic effects. However, it is not unlikely that activity of endogenous TRH accumbal system counteracts in the long term, the anxiety levels of DIA rats, as is proposed for different TRH-induced actions [101]. It is also possible that this increased expression of accumbal TRH, in DIA animals, leads to higher release of the peptide in TRHergic neurons' synaptic terminals of anxiety-regulating regions such as central amygdala and reducing anxiety-like behavior [88].

We injected i.p. two doses (0.2 and 2 mg/Kg of B.W.) of the PDE7 inhibitor to DIA animals trying to evince its potential anxiolytic effect and mediation of accumbal TRH expression changes. Figure 4 shows that both doses injected in DIA group, 24 h before performing the DBT, returned anxiety levels to control values. Results showed that male DIA rats injected with either dose of the PDE7 inhibitor spent less time burying the electric prod than controls (Figure 4). However, no further increase on accumbal TRH mRNA levels was observed with any dose (Figure 3).

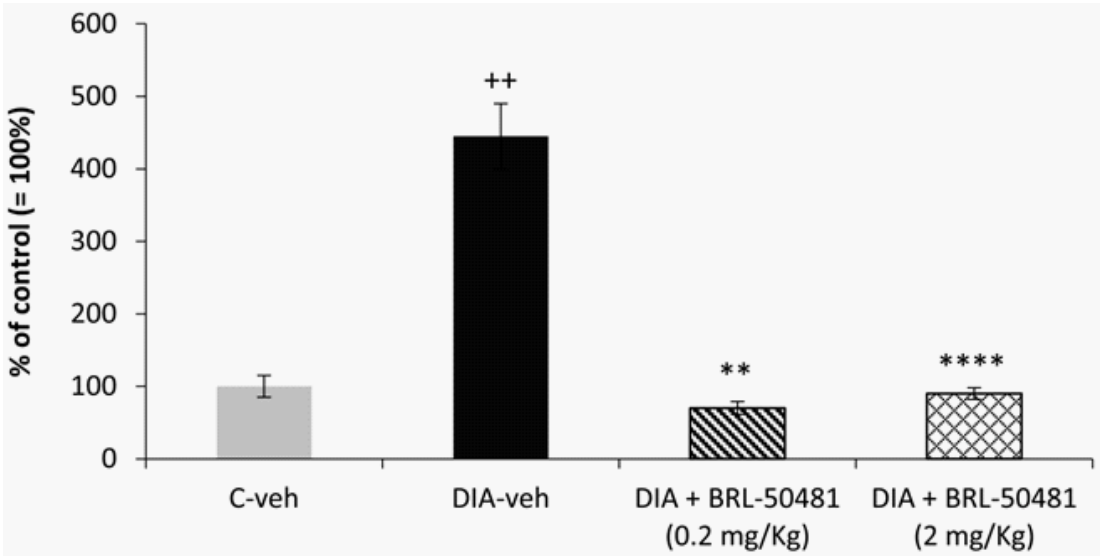


Figure 4: Anxiety-like behavior in control and DIA animals injected i.p. with vehicle (**veh**) or two doses (0.2 or 2 mg/ Kg of B.W.) of PDE7B inhibitor (BRL-50481) 24 h before their performance in the DBT. Values are the mean $\pm$ SEM of the time spent burying the electric prod in seconds, during 10 min of the test, and expressed as percentage of control values (=100%). One-way ANOVA, followed by a Fisher's post-hoc test showed significant differences when ++ p <0.01 vs. C-veh; ** p <0.01, **** p <0.0001 vs. DIA-veh. **C:** control, **DIA:** dehydration-induced anorexia.

When we analyzed food intake of DIA animals before and after being injected i.p. with the PDE7B inhibitor, we observed that the lower dose was effective to reduce anxiety with no change in feeding vs DIA-veh (Figure 5). Clinical studies are needed to propose this inhibitor as a therapeutic agent in anxious patients that present anorexia.

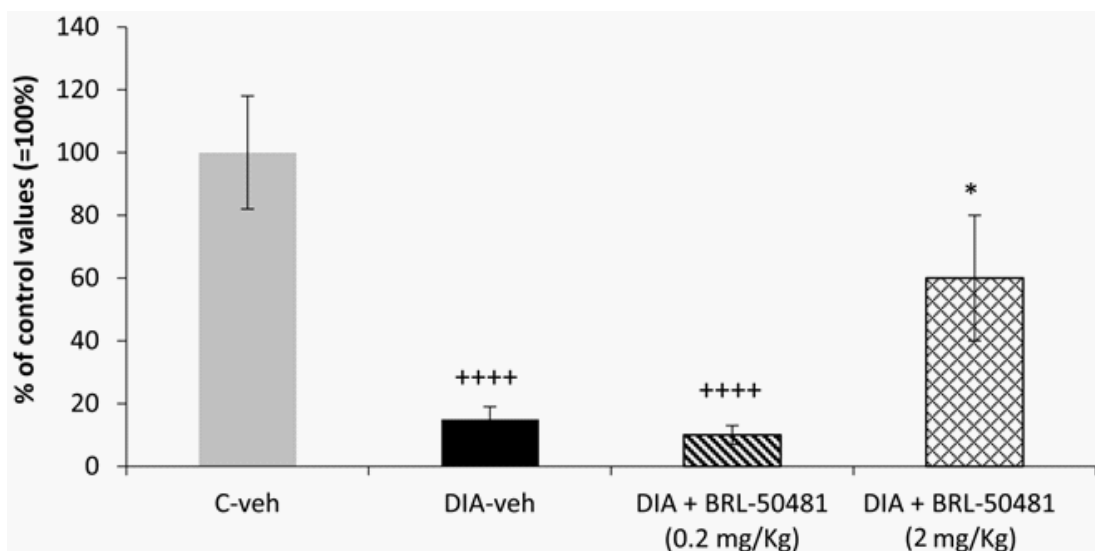


Figure 5: Food intake of control and DIA animals injected i.p. with vehicle (**veh**) or two doses (0.2 or 2 mg/Kg of B.W.) of PDE7B inhibitor (BRL-50481). On day 5, DIA food intake was registered during 1 h after animals were administered and before performing the DBT. Values are the mean $\pm$ SEM of grams of food and expressed as percentage of control values (=100%). One-way ANOVA, followed by a Fisher's post-hoc test showed significant differences when **** $p < 0.0001$ vs. C-veh; * $p < 0.05$ vs. DIA-veh. **C:** control, **DIA:** dehydration-induced anorexia.

In contrast, the higher dose of the PDE7B inhibitor also reduced anxiety levels but induced a slight increase in feeding. These dose-dependent effects of the inhibitor in feeding would be suitable for treating anxiety and modulating feeding behavior conveniently to fulfill patient needs. Further analyses of TRH protein levels in NAcc of anorexic rodents as well as, afferent projections of those neurons will help to understand its participation in regulation of mood and feeding disorders in anorexia.

Anorexia and depression

Depression is another important and frequent comorbidity of AN, occurring in approximately 80% of patients suffering from an eating disorder [102]. Clinical studies confirm that depressive symptoms may be the sequel of malnutrition in AN [103]; on the other hand, depression itself is a known predisposing factor for AN [104].

Anorexic behavior is not only characteristic of depression but also of hypothyroidism, thyroid hormone treatment has been proved as a successful therapy of some cases of refractory depression [105]. The most recognized disturbance in the hypothalamic-pituitary-thyroid axis (**HPT**) during depression is a lack of response of thyrotropin (**TSH**) to TRH stimulation, and it occurs in 25-30% of depressed patients [106]; thus, there might be a degree of central hypothyroidism

in some depressed patients. There is evidence for HPT axis chronobiological deregulation in major depressed patients [107], which is restored after 2 weeks of antidepressant treatment. Restoration of the HPT axis activity precedes clinical remission of depression and alterations of the HPT axis are associated with treatment resistance [108].

TRH is generally associated with regulation of the HPT axis but it also functions as a neuropeptide in certain key areas of the brain involved in mood regulation. For example, TRH from amygdala seems to mediate the depression-induced effects of stress [109]. Antidepressants have direct effects on hypothalamic TRH neurons, the ones from the tricyclic and serotonin reuptake families reduce TRH secretion [110]. In contrast to previous reports, TRH synthesis increases in hypophysiotropic neurons due to high cortisol levels frequently seen in depression [111].

The FST was developed as a model for predicting the clinical efficacy of antidepressants [112]. This test is useful to assess depression-like behavior in rodents; immobility becomes present as the test session progresses and it can be interpreted as behavioral despair [112]. It is noteworthy that behavioral and biochemical characteristics of animals in a state of despair during the FST, points to a useful animal model of stress and depression itself [113,114].

Antidepressants co-administration therapy increases the activity of cAMP pathway more rapidly than with the use of single drugs; this pathway is normally activated during chronic treatment of single compounds [115]. Rolipram is an inhibitor of the phosphodiesterase type 4 (**PDE4**), which has antidepressant effects, reducing immobility of rodents in the FST [116,117]. PDE4 family is encoded by four genes (PDE4A-PDE4D), and each isoform has multiple variants [92]. Subtypes 4A, 4B and 4D are widely distributed in the central nervous system [118,119], but interestingly, the strongest PDE4B immunoreactivity is detected in the NAcc [118].

Dopaminergic neurons from the *substantia nigra* are known to express PDE4B and PDE4D. Inhibition of PDE4 by rolipram increases tyrosine-hydroxylase phosphorylation in Ser40 protein kinase A (**PKA**-site) in nigrostriatal slices and *in vivo* [120]. Rolipram increases DA synthesis without altering its release and inhibits DA receptor type 2 signaling [121]. Such decrease on DA signaling may promote an increase in food intake given that DA is known to interact with the melanocortinergic system in the midbrain [122,123]. Inhibition of PDE4 also enhances cAMP/PKA signaling in striatal neurons [120]. Rolipram also exerts antidepressant-like effects presumably via induction of brain-derived neurotrophic factor (**BDNF**) and hippocampal neurogenesis [124].

Rolipram increases cAMP levels in rodent cerebral cortex and hippocampus; its effects are mediated mainly through modifications in glutamatergic neurotransmission, and it increases proliferation and neuroplasticity changes in hippocampus [117,125,126]. Studies conducted in our laboratory found that rolipram was able to reverse depression in anorexic animals (data not shown). A possible mechanism by which rolipram could be enhancing mood in DIA rodents is through modulation of TRH neurons from cerebral cortex and hippocampus due to the association of these brain regions with mood disorders.

Hippocampus is a region critically associated with depression and TRH in this region seems to be involved in the mood-altering effects of lithium administration and withdrawal frequently observed during treatment for depression [127]. Cerebral cortex is another brain area that seems to be responsive to the effects of rolipram administration and FST. Cortical TRH modulates synaptic transmission, and neuronal excitability [128,129] including the regulation of action potential shape in pyramidal neurons, in this way influencing information processing in the cortical network [130].

DIA is able to increase TRH expression in hippocampus, while it does not affect peptide's mRNA levels on cerebral cortex (Figure 6,7). When these animals were subjected to the FST, TRH levels in the hippocampus were no longer increased in comparison to controls and were even lower than those of DIA animals not subjected to the test. The contrary is observed in cerebral cortex, where TRH expression showed a tendency to be increased in both control and DIA animals subjected to the FST.

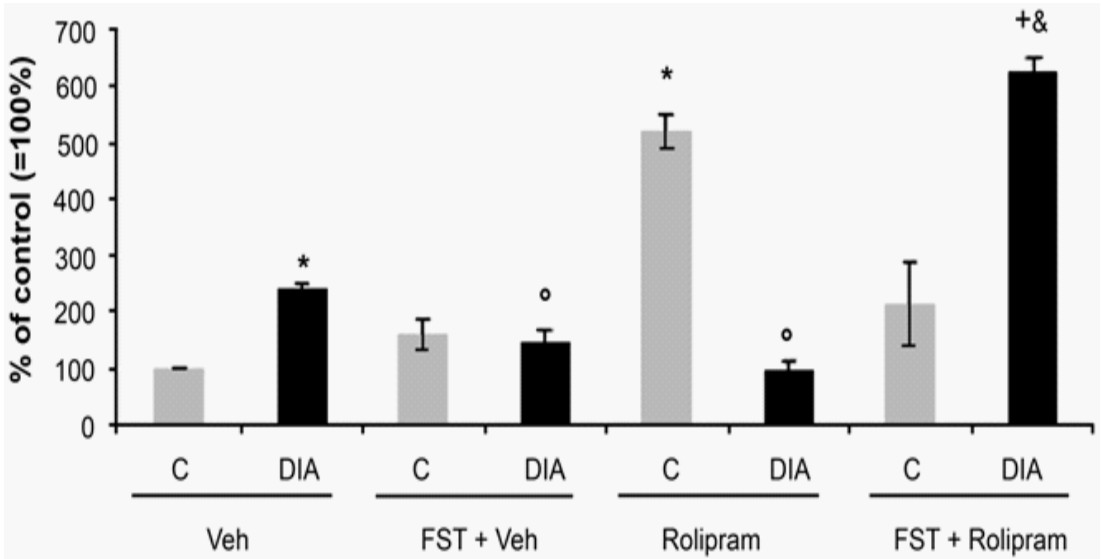


Figure 6: TRH mRNA expression in hippocampus of control and DIA rats. Animals were subjected or not to FST with or without administration of PDE4 inhibitor (rolipram). Data are expressed as mean $\pm$ SEM of arbitrary units of the ratio cDNA of TRH/cDNA of cyclophilin evaluated by RT-PCR and expressed as percentage of control values (=100%). **C:** control; **DIA:** dehydration-induced anorexic rats; **FST:** forced swim test; **Veh:** vehicle. Three-way ANOVA followed by post-hoc Tukey test showed significant differences when * $p<0.05$ vs. C, ° vs. DIA; + vs. DIA + Rolipram; & vs. DIA + FST.

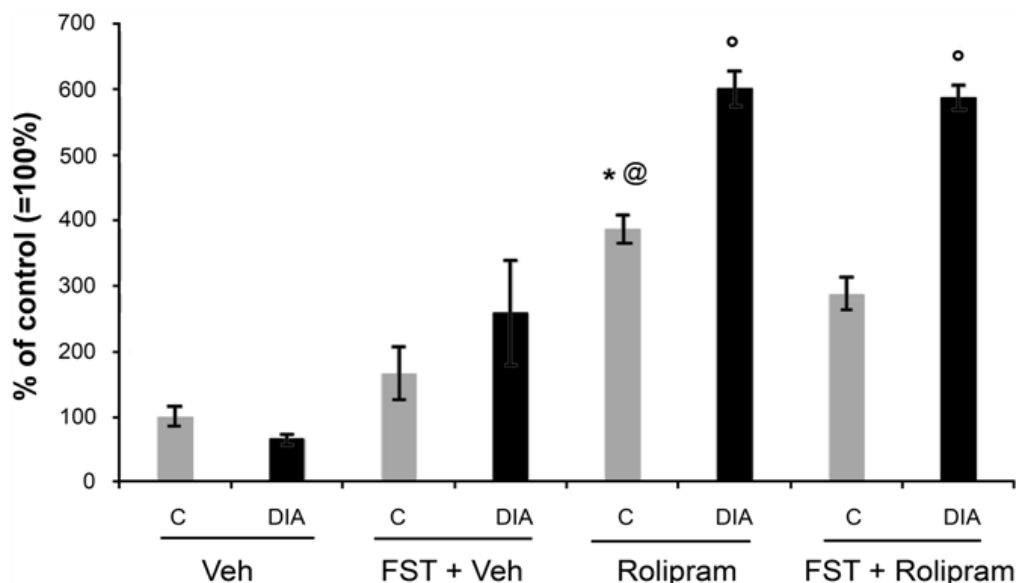


Figure 7: TRH mRNA expression in cerebral cortex of control and DIA rats. Each group was subjected or not to FST with or without administration of PDE4 inhibitor (rolipram). Data are expressed as mean $\pm$ SEM of arbitrary units of the ratio cDNA of TRH/cDNA of cyclophilin evaluated by RT-PCR and expressed as percentage of control values (=100%). **C:** control; **DIA:** dehydration-induced anorexic rats; **FST:** forced swim test; **Veh:** vehicle. Three-way ANOVA followed by post-hoc Tukey test showed significant differences when * $p < 0.05$ vs. C; @ vs. C+FST+Rolipram; ° vs. DIA.

Rolipram administration in DIA rats was able to increase TRH expression in hippocampus, only when they were evaluated in the FST, which might itself cause an acute stress response. In cerebral cortex, rolipram increased TRH expression to very high levels without an apparent effect of FST. The increase in TRH synthesis appears to be related to a decrease of PDE4D expression in this brain area. Overall, activation of TRHergic neurons in specific brain areas may underlie some of the mood enhancement actions of rolipram, a response that appeared to be related with stress in the hippocampus but not in cerebral cortex.

Comorbidity prevalence between mood and eating disorders in obese and anorexic patients is high. Treatment is problematic, since drug therapies mostly used are able to reduce anxiety but undesirably increase food intake in overweight individuals. In addition, anorexigenic drugs may induce anxiety or depression in individuals with eating disorders.

Animal models have been helpful to identify different neurotransmitter and neuropeptides' pathways that may regulate mood and food intake independently or simultaneously, as well as the brain regions involved in those processes. In this review, we are showing novel results related to the mediation of TRH-synthesizing brain regions in the potential use of PDEs inhibitors to reduce anxiety and depression in animals with dehydration-induced anorexia. Since TRH expression was not always related to changes in those behaviors in the studied brain regions, also different neuropeptides' systems should be analyzed.

Despite the existence of animal models that assess anorexia and mood disorders, a model where genetic and environmental factors interact to induce mood and feeding alterations is needed in order to gain a more accurate approach of the disorders as they are observed in humans.

ACKNOWLEDGMENTS

This study was supported by grant CONACyT 128316 (P. de Gortari). We thank to Dr. Erika Estrada for her assistance during behavioral tests' performance; to Jose Luis Calderón and Raúl Cardoso for their help with artwork.

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