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Associations of low testosterone levels with stress vulnerability and antidepressant response in aging males

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ABSTRACT

Increase in life expectancy worldwide is associated with a higher prevalence of stress-linked neuropsychiatric illnesses such as depression. Age-related alterations in neuroendocrine axes could contribute to the appearance of affective disorders and changes in response to conventional antidepressants. This review analyzes, in aged male rats, the role of testosterone in stress vulnerability, response to citalopram, and modulation of the expression of its pharmacologic target: brain serotonin transporter. In comparison with younger males, older hypogonadal males are more vulnerable to the chronic mild stress paradigm because they express higher rates of anhedonia, an effect that is prevented by restitution with testosterone. Resilience to chronic mild stress induced by testosterone seems to be associated with its inhibitory actions on hypothalamus-pituitary-adrenal axis function. In contrast with young males, aged males have reduced levels of serotonin transporter and exhibit slower antidepressant response to citalopram in chronic mild stress. Interestingly, testosterone restitution in older rats increases serotonin transporter expression in dorsal raphe

RESUMEN

Las alteraciones de los ejes neuroendocrinos asociadas al envejecimiento podrían contribuir a la aparición de trastornos afectivos y a cambios en la respuesta a los antidepresivos convencionales. Esta revisión analiza, en ratas viejas, el papel de la testosterona (T) en la vulnerabilidad al estrés, la respuesta al citalopram y la modulación de la expresión de su blanco farmacológico: el transportador de serotonina (SERT). En el paradigma de estrés crónico moderado, los machos viejos hipogonadales son más vulnerables al estrés que los jóvenes, puesto que tienen una mayor tasa de anhedonia; la expresión de la anhedonia fue prevenida con un tratamiento de restitución con T, posiblemente a través de un mecanismo de inhibición del eje hipotálamo-hipófisis-adrenal. Por otra parte, los machos viejos tienen menor expresión del SERT y muestran un retardo en la respuesta antidepresiva al citalopram; la restitución con T previno el déficit de SERT en el rafe dorsal. Los resultados sugieren la importancia de analizar el papel del hipogonadismo en la etiología de los trastornos afectivos. También sugieren que el

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nucleus, suggesting that this androgen plays a regulatory role in the actions of serotonergic antidepressants. Experimental studies lead to regard hypogonadism as a factor in the etiology of affective disorders and suggest that testosterone restitution should improve antidepressant response in later-life depression. (REV MEX ENDOCRINOL METAB NUTR. 2015;2:85-94)

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tratamiento de restitución con T podría mejorar la respuesta a antidepresivos en varones con depresión de inicio tardío.

Palabras clave: Envejecimiento. Antidepresivos. Depresión. Machos. Vulnerabilidad al estrés. Testosterona.

INTRODUCTION

Dysfunction of gonadal hormones by natural or pathologic causes is frequently accompanied by psychopathologies. Deficiency of androgens in aged men is associated with alterations in mood, irritability, and anxiety, which may lead to complex syndromes such as late-onset depression. Older people also show a poor response to antidepressant drugs[1]. However, the mechanisms underlying depression in aged men have not been elucidated. This review focuses in the analysis of some of these mechanisms found from the chronic mild stress paradigm (CMS), a validated test that reflects anhedonia, a core symptom of depression.

AGING IS A RISK FACTOR FOR DEPRESSION IN MEN

Depression is one of the most common, costly, and severe psychopathologies worldwide, characterized primarily by persistent low mood and anhedonia (lack of pleasure in usually enjoyable activities) plus symptoms that may include changes in sleep, feelings of guilt or worthlessness, low energy, poor concentration, changes in appetite, psychomotor retardation, and thoughts of death or suicide, which results in significant impairment in everyday living[2].

The biological bases of depression are complex and likely involve multiple interacting disruptions affecting neurons and glial cells within specific brain areas, giving rise to neural network dysfunctions and depressive symptomatology. Biological processes implicated in depression including altered monoaminergic neurotransmission, reduced neurotrophic support, immune reaction, oxidative stress, altered stress hormone homeostasis, and changes in levels of steroid hormones. Interestingly, biological pathways associated with depression overlap with those frequently implicated in aging processes. Notably, chronic stress, a common precipitating factor in depression, has been suggested as a factor leading to accelerated aging[3].

Depression is located at position 15 in the list of diseases with higher prevalence in men over 50 years of age[4]. In the elderly, depression is the most common psychiatric disease, with a prevalence ranging from 22 to 46% in patients over 65 years old[5]. This observation acquires relevance since the world´s geriatric population is importantly increasing[6]. These data suggest that aging represents a risk factor for developing depression.

Aged people are primary treated with selective serotonin reuptake inhibitors (SSRI)[7]; however, it has been observed that the treatment is longer in aged than in young patients[1,8] and, in some cases (18%), aged patients suffer depression refractory to antidepressants[9]. For this reason, clinical research has been focused in new treatment algorithms to improve symptoms of depression. However, more investigation is needed on possible mechanisms underlying this clinical issue.

AGING REDUCES SECRETION OF ANDROGENS

Aging is the process of progressive decline in the biological functions of cells and organs that causes most organisms to suffer from exponentially increasing mortality rates over time[10].

Human aging is associated with a decrease of circulating gonadal steroid hormones. This is associated with the progression of neurodegenerative disorders, increased depressive symptoms, and other psychiatric disorders[11].

Two of the most important hormonal changes related to aging in males are a gradual reduction in testosterone (T) levels and a stress-dependent sustained increase of plasmatic glucocorticoids due to an impaired negative feedback in the hypothalamic-pituitary-adrenal axis (HPA). Interestingly, these hormonal changes have also been related to depression[12]. Another important change found in human aging is the 5- to 10-fold decrease in levels of dehydroepiandrosterone (DHEA), a steroid secreted by the adrenal glands[13]. Higher levels of this hormone have been linked with better health outcomes and one study has found a correlation between high levels of DHEA and increased longevity in males[14].

LEVELS OF TESTOSTERONE IN AGING

Testosterone is the primary androgen secreted by testes. Pulsatile secretion of gonadotropin-releasing hormone regulates pituitary secretion of luteinizing hormone, which stimulates testicular production of T, which is converted by the intracellular enzyme 5α-reductase into dihydrotestosterone (DHT), a more potent ligand for the androgen receptor. Testosterone is also converted into estradiol (E2) by the enzyme aromatase in fat, skeletal muscle, and other tissues. Therefore, actions of T can be mediated by T or DHT acting via the androgen receptor or by E2 acting via the estrogen receptor (ER)-α or -β[15].

Approximately 98% of T is bound to sex-hormone-binding globulin (SHBG) and other serum proteins, including albumin as free T; this fraction together with that bound to albumine are referred as bioavailable T. This is generally considered the biologically active portion of T[16], deficiency of this hormone (hypogonadism) is a widely recognized hormonal alteration associated with male aging. Laboratory diagnosis of hypogonadism is based on the measurement of serum total T: levels above 350 ng/dl are considered normal and do not require substitution therapy, while T levels below 230 ng/dl usually benefit from T treatment[16]. Age-related serum T decline is caused by different simultaneous mechanisms, such as primary structural gonadal impairment, degenerative modifications of the pituitary gland, deficits of the neurohypothalamic system, and primary peripheral metabolic abnormalities such as the increase in the concentration of serum SHBG, with a consequent decrease in free T[17].

In aging, total and free T levels decline at a rate of 0.4% and 1% per year, respectively. Meanwhile levels of SBHG increase aproximately 1.2% per year, increasing the binding of T to SHBG; increase at a rate of approximately 1.2% per year, increasing the binding of T to SHBG; this provides an explanation for the decline in free T levels. Also, the reduction of T in aging has been associated with a decreased Leydig cell function and hypothalamic-pituitary-gonadal axis sensitivity, so aged men have more difficulty to compensate the reduction of T[12].

The decline in circulating levels of T may be associated with changes in somatic and psychological features in men, such as decline in libido, erectile dysfunction, increased fat deposition, decreased muscle mass, decreased energy, and depression. The relationship between increased depressive symptoms and declining T levels is complex because many conditions are independently associated with depression and T deficiency, as are genetic, environmental, and personality factors. Many studies have demonstrated the improvement in depressive symptoms in hypogonadal men with T supplementation[18]. Besides, it is unclear whether hypogonadism causes depression, increases stress vulnerability, or leads to resistance to standard antidepressant treatments[12].

AGING AND HYPOGONADISM INCREASE STRESS VULNERABILITY

Stress is defined as the nonspecific response of the body to any demand[19,20]. This response is induced by any new situation (chemical, physical, environmental, emotional, and psychosocial stressor), which disturbs the homeostasis and induces a general adaptive response aimed to restore the initial level

of stability (adaptation). All the manifestations of the adaptive response are beneficial to the organism when limited in time, but when the duration of the stress is excessive, it contributes to the development of pathological conditions[19,20].

It has been described that aging is characterized by a decreased ability to maintain homeostasis and consequently less efficient adaptation to change[21], and thus it becomes a potent vulnerability factor for the impact of aversive stimuli. Mroczed and Almeida[22] reported that the association between daily stress and negative affect (reactivity) was stronger among old than young adults.

It is known that chronic stress (ongoing for weeks or months) is a stronger predictor of depressive symptoms than acute stressors[23] and multiple stressful events substantially increase the risk of depression onset[24]. A widely-used animal model to study depression is the chronic mild stress (CMS)[25]. This paradigm induces anhedonia by exposing rats to a chronic period (5-9 weeks) of mild and unpredictable environmental stressors. Commonly, the stress protocols include periods of food and water deprivation, continuous lighting, cage tilt (30°), paired housing, soiled cage (100 ml water spilled onto bedding), exposure to reduced temperature (10°C), intermittent white noise (85 dB), stroboscopic lighting (300 flashes/rain), exposure to an empty water bottle following a period of water deprivation, restricted access to food (scattering of a few 45 mg precision pellets in the animal's home cage), novel odors (e.g. fresh air deodorant), or presence of a foreign object in the home cage, (e.g. piece of wood or plastic). The anhedonia, which is a central feature of endogenous depression, is evaluated in this model by the reduction of the palatable sucrose solution consumption[25].

It was shown that aged (12-15 months) rats were more likely to develop anhedonia after exposure to the CMS battery compared to young (3-5 months) rats[26,27]. In our previous study[27], young adult and middle-aged male rats were exposed, during three weeks, to a CMS schedule including several stressors: white noise, overcrowding, continuous light, soiled cage, stroboscopic light, water deprivation, cage tilt, and food deprivation. We found that a high proportion of aged animals reduced their sucrose solution consumption a few weeks after CMS, indicating that these rats are more susceptible to stress than young animals. This increased vulnerability may be associated to low T levels since this hormone was importantly reduced in middle-aged rats (74%) as compared to young adults[27]. This situation seems to be age-dependent because depletion of androgens by castration did not increase susceptibility of young males to the CMS[28].

With the aim of evaluating the preventive effect of T restitution on stress vulnerability of middle-aged male rats, we administered the hormone (using a pellet with T propionate [9 mg] placed in the rats' cervical region) three weeks before exposure to CMS. This method of restitution maintained the T levels of middle-aged rats in the range of those found in young adults rats (3.97 ± 1.09 ng/ml) during four weeks[28]. Results indicated that the administration of T prevents the development of anhedonia produced by CMS in aged animals. These data suggest that T is an important factor that favors resiliency (capacity of an organism to successfully adapt to adversity) by regulating the negative effects of stress. In agreement with this idea, Bernardi, et al.[29] found that castration significantly increased the depressive-like behaviors in two animal models whose behavioral deficits were evoked by acute stress, the tail-suspension and the forced swimming test. In turn, administration of T propionate (1 and 10 mg/kg subcutaneously during four days) decreased the depressive-like behaviors in castrated mice.

From the evidences here presented it could be concluded that aged subjects, with low levels of gonadal steroids, are more sensitive to the negative effects of stress (which has been related with an increased HPA axis activity) and this, in turn, may cause a higher susceptibility to developing depression.

TESTOSTERONE DOWN-REGULATES ACTIVITY OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

Vulnerability or resilience to stress is related to an inadequate or adequate regulation of the HPA axis, respectively. In this regard, the diathesis-stress hypothesis of depression states that chronic stress could

induce physiological alterations in vulnerable subjects that promote an exaggerated stress response, which in turn would cause depressive events[30,31]. Accordingly, corticosterone levels in the stressed anhedonic middle-aged rats were higher than those of control rats; this difference was blunted by T restitution (Herrera-Pérez, unpublished data). These effects could be attributable, at least in part, to the inhibitory role of this hormone on HPA axis activity. Several studies evidence this modulation; the first one indicates that HPA axis in female rodents is more active than in males when exposed to several stressors, as reflected by glucocorticoid and adrenocorticotrophic hormone (ACTH) levels[32-34]. It has also been observed that androgen (T and DHT) administration in rodents reduced secretion of glucocorticoids, ACTH, and the stress-induced expression of *c-fos* in the hypothalamus[32,35,36]. In line with this, several reports indicate that orchiectomy disinhibits the response of HPA axis when male rats are challenged with stressors; this activity is restored after androgen treatment[36,37].

The inhibitory effect of androgens on HPA activity has several mechanisms:

- reducing hypothalamic levels of corticotropin releasing factor and ACTH content in the pituitary, corticosterone levels, and adrenal gland weight[38,39];
- increasing the expression of glucocorticoid receptors in the hippocampus, improving the negative feedback of the HPA axis[40];
- improving neuronal activity in hippocampus and lateral septum (two structures that inhibit HPA activity) of rodents exposed to a stressor[41];
- inhibiting the enzymatic activity of 11-beta hydroxysteroid dehydrogenase 1, an enzyme that converts dihydrocorticosterone to active corticosterone[42].

AGING REDUCES RESPONSE TO ANTIDEPRESSANTS IN MALES

Results in the CMS paradigm suggest that antidepressant response to SSRIs is modulated by age. Adult young male rats treated with citalopram (10 mg/kg) showed an increase in sucrose solution intake that started after one week of treatment, peaked at the second, and stabilized at the third. Middle-aged rats exhibited a delay in response to treatment since the sucrose solution intake increased at the third week of treatment, and the magnitude of the antidepressant-like effect was mildly lower than that of young adults[43]. Similarly, Bourin, et al.[44,45] described that four-week-old mice showed a consistent antidepressant-like behavior in the forced swimming test with a variety of SSRIs (sertraline, paroxetine, citalopram, fluvoxamine), tricyclics (desipramine, imipramine, maprotiline), and third-generation antidepressants (venlafaxine), while 40-week-old mice did not respond to citalopram and paroxetine[44,45]. These results are in line with meta-analysis data[46], which reveal a better efficacy of tricyclics than SSRIs in older depressed people (> 65 years old). Interestingly, citalopram is chosen as a first-line treatment in the elderly on the basis of its efficacy, safety, and selectivity for the serotonin transporter (SERT)[47].

The study of David, et al.[45] proposes that aging affects serotonergic receptors that participate in the response to the most important clinically used antidepressants. For elucidation of a possible mechanism, young and old mice were pre-treated with buspirone, a partial 5-HT1A agonist, or anpirtoline, a 5-HT1B agonist, and later treated with SSRIs, tricyclics, or dual antidepressants (with 5-HT and noradrenaline reuptake actions). The results indicated that in the 40-week-old mice, buspirone facilitated the effects of antidepressants, while anpirtoline was unable to synergize with SSRIs, suggesting that the elderly have lower 5-H1B receptor functionality. This result agrees with the suggestion of Gozlan, et al.[48] that the density of 5-HT1B decreases with aging but that of 5-HT1A does not change.

Human studies that associate depression in the elderly with antidepressant efficacy have not analyzed the impact of sex on clinical response[46,49]. For this reason, animal research could serve as a starting point for this comparison. Data from CMS reveals important differences because ovariectomized middle-aged rats fail to exhibit a delay in response to citalopram (10 mg/kg), which was produced from the first week of treatment[50]. Additionally, sub-threshold doses of fluoxetine produce in these females a tendency to improve sucrose intake from the second week of treatment, which reached a complete anti-anhedonic effect

in combination with estradiol treatment. Middle-aged females treated with this combination also showed an earlier antidepressant response in comparison to gonadally intact younger rats[51]. In these studies, middle-aged rats were ovariectomized and their treatment started three weeks after surgery, and therefore they were completely depleted of estrogens at the starting of treatment. This suggests a sex difference in modulation of serotonergic system, associated to low levels of gonadal hormones. In old males, partial deficiency of androgens seems to account for a more severe dysfunction of this system with repercussions for therapy with psychotropic drugs.

AGING MODULATES BRAIN SEROTONIN TRANSPORTER EXPRESSION: IMPLICATIONS FOR ANTIDEPRESSANT RESPONSE

The differential response to antidepressant treatment in young and middle-aged rats may suggest that the dose of citalopram was not enough to reach the pharmacological effect in middle-aged animals. However, this hypothesis is discarded for several reasons: the enzymatic complex activity that metabolizes citalopram in the liver (the cytochrome P450 enzymatic complex)[52] is reduced by aging[53], and renal excretion is impaired in the elderly[47]. Additionally, hypogonadism in old males would contribute to increase citalopram blood concentrations by a reduction in its liver demethylation rate, which is regulated by T[54]. These conditions imply that the citalopram plasmatic concentration is higher in aged animals than in the young after receiving the same dose. Thus, pharmacokinetic changes associated to aging fail to explain the impaired response to citalopram in middle-aged rats.

Alternatively, the blunted response to citalopram in middle-aged rats could be based on the age differences in brain SERT expression, the molecular target for citalopram, a hypothesis that was tested in our laboratory. The brain SERT was quantified by immunofluorescence in young adult and middle-aged rats[55]. The evaluation was done in several brain areas related to depression or its treatment: prefrontal cortex, hippocampus, lateral septum (structures able to modulate the activity of the HPA axis)[56,57] and the raphe nuclei (containing the cellular bodies of serotoninergic neurons). The study indicated that these brain structures of middle-aged rats had lower SERT expression than their young counterparts[55], a result that is in agreement with those found in Rhesus monkeys[58]and humans[59,60].

Because SERT immunoreactivity correlates with serotonin immunoreactivity in rat brain, it may be an indicator of serotonergic innervation[61-64]. Thus, the reduction in brain SERT expression associated to aging implies a general impairment of serotoninergic neurotransmission. This idea agrees with the aberrant serotoninergic fibers found in the brain of aged rats[65], the reduced binding potential of [11C](+) McN5652 to brain SERT in aged humans[60], and the low brain serotonin levels associated with aging[66].

These data, together with the idea that the antidepressant effect of a SSRI depends on the blockade of an adequate number of SERT[58,67], support the hypothesis that the low SERT expression in middle-aged animals accounts for their retarded response to citalopram. Thus, compared to the young, citalopram administration to middle-aged rats would inhibit a reduced number of SERTs, originating lower serotonin availability in the synaptic cleft. In this way, the suitable concentration of synaptic serotonin needed to trigger the pharmacological effect would be reached in a longer time. In agreement, clinical studies suggest that a high availability of SERT in the diencephalon before treatment predicts a better response to treatment with a SSRI[68] and that depressed patients that carry the short isoform of the SERT gene regulatory region (associated with a low SERT expression[69-71]) responded deficiently to this treatment[67,72,73].

According to Lipsitz and Goldberger[74], "aging is a process in which the complexity of biologic systems is reduced, making it difficult for the aged individual to cope with external or internal disturbances". For the central nervous system, this would be related with a reduction in complexity of neural networks, as suggested by studies that show a reduction of neural arborization in the cortex[75,76] and the hippocampal CA1 region[77]. In agreement, the study from our laboratory suggests that aging reduces the density of the serotoninergic fibers in prefrontal

Table 1. Changes in central neurotransmission associated to aging

	Change	Reference
Brain serotonin levels	Decrease	(66)
5HT1D and 5HT2 receptors in frontal cortex	Decrease	(92)
Dopaminergic D2 receptors	Decrease	(93)
Noradrenaline in hypothalamus	Decrease	(94)
Noradrenergic neurons in *locus coeruleus*	Decrease	(95)
Brain MAO A	Increase/no change	(96)
Brain MAO B	Increase	(97)

MAO: monoamine oxidase.

cortex, lateral septum, hippocampus, and raphe nuclei[55]. Aging also reduces other parameters of neurotransmission systems, as indicated in table 1. These age-related alterations of central neurotransmission have an impact in response to other psychotropic drugs, for example tricyclic antidepressants[45] and anxiolytics[78].

The neural network theory states that depression is determined by the loss of communication among neurons and that antidepressant treatment increases such communication, achieving remission of depression[79]. Following this idea, depression is reversed only when the individual has mechanisms of brain plasticity able to respond to antidepressant treatment[80,81]. Several studies indicate that aged animals have low brain plasticity, as suggested by their low expression of brain-derived neurotrophic factor (BDNF)[82] and reduced neurogenesis in the hippocampal dentate gyrus[83]. This condition is behaviorally manifested in the impaired response of aged rats in memory tests[84]. In turn, increased neurogenesis has been proposed to underlie important behavioral effects induced by antidepressants[85]. Thus, the reduced brain plasticity also would challenge the response of aged rats to antidepressant treatment.

MODULATION OF SEROTONIN TRANSPORTER BRAIN EXPRESSION BY TESTOSTERONE

The influence of aging on neurotransmitter systems could be explained, at least in part, by hormonal changes (as is the case of gonadal hormones) presented in aged individuals; these steroids have several effects on neurotransmitter systems and thus have effect on cognitive and affective functions. Testosterone, for example, reduces monoamine oxidase activity[86], and in this way, this hormone facilitates the monoaminergic neurotransmission. In the specific case of serotoninergic system, it has been described that T and E2 are able to regulate the expression of serotoninergic receptors 5HT1A and 5HT1C in several brain regions[87], and these hormones also have the ability to modulate SERT expression[88]. These authors found that castration of male young rats reduced SERT expression in dorsal raphe, a result that supports the idea that the age-related reduction in T levels[27] is responsible for a deteriorated serotoninergic system.

If the impaired response of aged males to antidepressants depends on the modulatory role of gonadal hormones on neurotransmitter systems, then T restitution would be able to modify the expression of therapeutic targets of antidepressants. This relationship was also studied in our laboratory. We found that T restitution in middle-aged rats increases SERT immunoreactivity in dorsal raphe as compared to intact middle-aged animals without restitution[55], a result that is in agreement with those found by McQueen, et al.[88] in castrated young adult rats treated with T or E2. These findings have therapeutic implications: raphe nuclei contain the somas of serotonergic neurons; in these nuclei, antidepressant treatment induces an important rise in serotonin levels needed to desensitize somatodendritic 5HT1A receptors. Such desensitization leads to an increase in

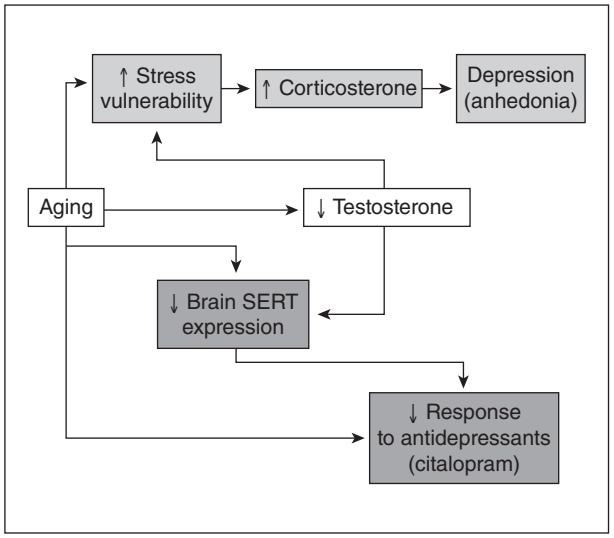

Figure 1. Associations of hypogonadism with stress vulnerability and antidepressant response in elderly. Diminution of testosterone in aging increases stress vulnerability, inducing the dysregulation of hypothalamic-pituitary-adrenal axis. This condition may produce higher rates of anhedonia, and reduce brain serotonin transporter expression that reduces antidepressant response.

the neuronal rate of serotonin release in the serotoninergic projections in all the brain, starting the antidepressant effect[89]. Testosterone could also facilitate the antidepressant response by increasing BDNF levels and hippocampal neurogenesis[82,83], a process implied in antidepressant response[80]. Besides, the role of T in pharmacologic effects is supported by basic studies where gonadal hormones restored the antidepressant-like effect of fluoxetine in orchidectomized young rats[90], and by clinical studies showing that T supplementation improved the antidepressant action of SSRIs in hypogonadal patients with major depression refractory to treatment[91].

CONCLUSION

The data here presented support the idea that aging is related to high vulnerability to stress and a poor response to antidepressant treatment, and both deficiencies may be explained by the reduced T levels found in aged animals (Fig. 1). The age-related reduction in T increases stress vulnerability, inducing the hypersecretion of corticosterone that produces anhedonia; besides, low hormonal levels reduce brain SERT expression that reduces the antidepressant response. Both effects may be prevented by T restitution.

These interpretations may be useful for the diagnosis, prognosis, and assignment of treatment in older depressed men. It could be important taking into account that: (i) hypogonadism should be studied as a vulnerability factor that increases the risk for developing affective disorders in middle-aged men; (ii) low levels of androgens should be analyzed in cases of depression refractory to serotonergic antidepressants or with delayed response; and (iii) T supplementation may be included as an adjunct treatment to conventional SSRIs in older men, considering the balance between benefits and risks of a hormonal therapy.

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