Review

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Dual role of T-type calcium channels in anxiety-related behavior

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T-type calcium channels are low voltage activated calcium channels that are widely expressed in various brain regions including stress-responsive regions. These channels regulate the diverse functions of the central nervous system, and modulation of these channels is shown to modulate the anxiety. Studies have described that modulation of T-type calcium channels may either aggravate or ameliorate anxiety-related behavior, suggesting the dual role of these channels. The studies employing animals with overexpression of T-type calcium channels reported their anxiety-inducing role. Therefore, the blockade of these channels using various pharmacological agents such as ethosuximide, plant extracts of linalool or rosemary, and corticotropin-releasing factor (CRF) is reported to ameliorate anxiety. On the contrary, knockout of the gene encoding these channels predisposes the rodents to anxiety-related disorders, suggesting the anxiety-attenuating role of these channels. It may be possible that these channels in normal or basal state attenuate anxiety, whereas activation of these channels in stressful condition may produce anxiety. The present review describes the dual role of T-type calcium channels in anxiety-related behavior in both preclinical and clinical studies.

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Introduction

Anxiety is a normal emotion that helps a person to be aware of forthcoming risk situations that may be real or imaginary. A persistent and increased level of anxiety is contemplated as a neuropsychiatric disorder that causes disturbances in the physical and mental health [1], [2]. According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, anxiety disorders include disorders having features of excessive fear and related behavioral disturbances. These disorders include separation anxiety disorder, social anxiety disorder (social phobia), panic disorder, agoraphobia, generalized anxiety disorder and anxiety disorders due to other medical conditions and medication/substance. According to two major studies in the United States-the Epidemiological Catchment Area study and the National Comorbidity Survey study, the estimated lifetime prevalence rates for individual anxiety disorders are as follows: 2.3%–2.7% for panic disorder, 4.1%–6.6% for generalized anxiety disorder and 2.6%–13.3% for social phobia [3]. In the United States, the approximate prevalence of the anxiety disorders is 3.1% and 5.7% over a patient's lifetime. Moreover, the prevalence of anxiety disorder is twice in women in comparison with men [4].

Calcium channels are broadly classified in to two types, voltage-gated calcium channels and ligand-gated calcium channels. Voltage-gated calcium channels are more prominent, and these respond to changes in the voltage across the membrane by allowing the entry of Ca^{2+} ions inside the cell, which are one of the main signal transduction elements in the neurons. These voltage-gated channels are further subdivided into two types: high voltage activated channels, consisting of L-type calcium channels, P/Q-type calcium channels and N-type calcium channels; and low voltage activated (T-type) calcium channels [5], [6], [7]. Previous studies have demonstrated that even a small membrane depolarization of the cell membrane is responsible for the activation of low-voltage activated T-type calcium channels [8], [9].

T-type calcium channels are expressed throughout the brain, especially in stress-sensitive regions of the brain including the amygdala, hippocampus, prefrontal cortex, temporal lobe and dorsal region of dorsal raphe

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nucleus [6], [10], [11]. T-type Ca^{2+} channels have been associated with a wide range of physiological processes such as sleep [12], neuronal firing, pain [13], [14] and motor coordination [15]. Disturbance in these channels leads to various neurological and neuropsychiatric disorders such as depression, memory impairments [16], cognitive deficits [17] and epilepsy [18]. Therefore, modulation of T-type calcium channels may be involved in the aggravation or amelioration of anxious symptoms [5], [19]. T-type calcium channels play a dual role in anxiety. Some studies have shown that overexpression of $\text{Ca}_v3.2$ T-type calcium channels produce anxiety-like behavior, and inhibition of activity of these channels shows anxiolytic activity [7], [20], [21]. Some studies have described that knockout of $\text{Ca}_v3.2$ T-type Ca^{2+} channels cause the development of anxiety-related behaviors [5], [22]. The present review describes the dual role of T-type Ca^{2+} channels in the anxiety-related behavior in preclinical and clinical studies using various behavioral tests and biochemical parameters.

Opening of T-Type Ca^{2+} channels induces anxiety-related behavior and T-type Ca^{2+} channel blockers attenuate anxiety

There have been studies documentixng that the opening of T-type Ca^{2+} channels produces anxiety and the blockade of these channels attenuates anxiety-related behavior. The study of Alaoui et al. suggests that the beneficial effects of two Mediterranean plants, i.e. lavender and rosemary, in nervous disorders such as anxiety and mood are mediated through the blockade of T-type calcium channels. It was shown that linalool and rosmarinic acid, the major active components present in the extracts of lavender and rosemary, respectively, modify the electrophysiological properties of recombinant T-type calcium channel ($\text{Ca}_v3.2$) expressed in HEK-293 T cells. These active components inhibited T-type calcium channels in a concentration-dependent manner and elevated a negative shift of steady-state inactivation of the $\text{Ca}_v3.2$ current, without showing any effect on activation properties [7]. The volatile compounds such as linalool and linalyl acetate that comprise lavender essential oil have demonstrated the therapeutic properties in disorders such as in anxiety in preclinical studies [23]. Furthermore, the anxiolytic activities of lavender have been attributed to linalool [24], and the latter is reported to attenuate stress-induced psychological parameters [25]. Moreover, lavender oil has been shown to reduce the calcium influx by modulating the voltage-dependent calcium channels [19]. Similarly, rosmarinic acid-containing plants such as *Rosmarinus officinalis* and *Melissa officinalis* also show an anxiolytic behavior [26]. Moreover, rosmarinic acid has also been reported to prevent anxiety-related behavior in cerebral-injured rats [27]. Accordingly, it has been proposed that the inhibition of T-type calcium channels could contribute to the anxiolytic effects of linalool- and rosmarinic acid-containing plants [7].

The report of Tao et al. suggests that the activation of corticotropin-releasing factor (CRF) receptor 1 by CRF peptides such as CRF or urocortin-1 selectively inhibits $\text{Ca}_v3.2$ T-type calcium channels. The CRF peptides including CRF and urocortins 1–3 are regulators of mammalian stress and are implicated in disorders such as anxiety [28], [29]. Mechanistically, it has been reported that the activation of CRF receptor 1 by either CRF or urocortin 1 reversibly and selectively inhibits $\text{Ca}_v3.2$ currents in HEK-293 T cells ($\text{IC}_{50} \approx 30$ nM) without modulating $\text{Ca}_v3.1$ or $\text{Ca}_v3.3$ channels. Moreover, the selective blockade of CRF receptors 1 using astressin abolished CRF-mediated inhibition of $\text{Ca}_v3.2$ channels in a significant manner. The CRF receptor 1-mediated inhibition of $\text{Ca}_v3.2$ T-type calcium channel was dependent upon the activation of cholera toxin-sensitive G-protein β_γ subunits ($G_{\beta\gamma}$). Accordingly, it may be proposed that CRF peptides may produce antianxiety effects by activation of CRF receptor 1, which in turn may inhibit $\text{Ca}_v3.2$ -type calcium channel using cholera toxin-sensitive $G_{\beta\gamma}$ signaling pathway [21]. Moreover, it has also been shown that the inhibitory effects of CRF-related peptide (urocortin 1) on T-type Ca^{2+} channels were abolished by specific CRF 1 receptor antagonist as well as by protein kinase C (PKC) inhibitors. Moreover, PKC activator (phorbol 12-myristate 13-acetate) mimicked the effects of urocortin and inhibited T-type calcium channels. It suggests that CRF/urocortin acts on CRF 1 receptors 1 to trigger PKC signaling pathway to inhibit T-type Ca^{2+} channels [30].

Ji et al. demonstrated the role of acute ethosuximide exposure in the modulation of the anxiety-related behavior in zebra fish larvae. The exposure of ethosuximide in low doses (2, 5 and 10 mmol/L) was reported to increase the locomotor response (distance travelled per minute). Moreover, it led to the amelioration of thigmotaxis behavior, suggesting its anxiolytic effects. On the contrary, high doses of ethosuximide (20 and 40 mmol/L) were reported to increase the thigmotaxis behavior and decrease the locomotor response, indicating that it results in the development of anxiety in high doses [31]. The study of Powell et al. demonstrated the difference in the pattern of anxiety-related behavior in two different strains of rats, i.e. genetic absence epilepsy rats (GAERS) and non-epileptic control (NEC) rats. It was shown that the GAERS displayed consistent anxiety and depressive-like behavior in comparison with the NEC rats. Mechanistically, a mutation was identified in the *cacna1h* gene, responsible for the expression of $\text{Ca}_v3.2$ T-type calcium channel, in the GAERS strain. Indeed, in this strain, the mutation in *cacna1h* leads to the overexpression of $\text{Ca}_v3.2$ T-type calcium channels, which

is associated with the development of symptoms of anxiety. This gene was normally expressed in the NEC rats, and these rats did not develop anxiety; therefore, it may be suggested that the mutation of the gene in the GAERS strain leads to anxiety and depressive-like behavior [20].

The study of Dezzi et al. also reported that chronic treatment with ethosuximide ameliorates epileptogenesis and behavioral comorbidity such as anxious behavior in the GAERS strain, a model of genetic generalized epilepsy. Animals (GAERS and NEC rats) were administered with ethosuximide (200 mg/kg, i.p.) in drinking water from the age of 3–24 weeks; after, that treatment was ceased, and all animals reverted to tap water, until the age of 34 weeks. The anxious behavior of the animals was assessed using an open field apparatus at the end of the 7th and 34th weeks. At the end of the seventh week, the distance travelled and the number of entries were significantly reduced in the open field apparatus in the GAERS as compared with the NEC rats, suggesting that the development of epilepsy/seizure leads to the induction of anxiety-related behavior. However, at the end of 34 weeks, the exploratory behavior was significantly restored in the ethosuximide-treated GAERS strain. It suggests that the upregulation of T-type calcium channels is responsible for the induction of anxiety and that its blockade may overcome anxiety-related behavior [32]. The study of Shaw et al. described the role of ethosuximide in the rat model of the absence epilepsy with anxiety as comorbidity, in Long-Evans rats. Anxiety-like behavior was assessed using open field and elevated plus maze tests. Acute administration of ethosuximide (100 mg/kg, i.p.) led to significant improvement in the behavioral performances, and the rats displayed higher frequency of line crossing along with longer stay period in the center zone of the open field apparatus. It may be suggested that acute blockade of T-type Ca^{2+} channels may ameliorate anxiety-related behavior associated with seizures [33]. However, a study of Munro et al. described that ethosuximide (30 or 100 mg/kg, i.p.) does not modulate the expression of anxiety-related behavior [34] (Table 1).

Table 1: Summarization of the dual role of T-type Ca^{2+} channels in anxiety-related behavior.

S. no.	Pharmacological treatment	Animals/cell lines	Function of T-type Ca^{2+} channels	Effect on anxiety	Reference
Inhibition of T-type Ca^{2+} channels ameliorate anxiety-related behavior					
1	Lavender and rosemary oil	Cell line assay (HEK293 cells)	Inhibition of T-type Ca^{2+} channels	Decrease in anxiety	[7]
2	Lavender oil	Mice	Inhibition of T-type Ca^{2+} channels	Decrease in anxiety-related behavioral alterations	[19]
3	Corticotropin-releasing factor peptides	Cell line assay (HEK293 cells)	Inhibition of $\text{Ca}_v3.2$ T-type Ca^{2+} channels	Decrease in anxiety	[21]
4	Ethosuximide (2, 5 and 10 mmol/L)	Zebra Fish	Blockade of T-type Ca^{2+} channels	Decrease in anxiety-related behavioral changes	[31]
5	NA	Genetic absence epileptic rats (GAERS)	Overexpression of $\text{Ca}_v3.2$ T-type Ca^{2+} channels	Consistent anxiety and depressive-like behavior	[20]
6	Ethosuximide (200 mg/kg)	Genetic absence epileptic rats (GAERS)	Blockade of T-type Ca^{2+} channels	Decrease in epilepsy-comorbid anxiety-like behavior	[32]
Genetic ablation of T-type Ca^{2+} channel aggravate anxiety-related behavior					
7	NA	Mice	Genetic ablation of <i>cacna1h</i> gene ($\text{Ca}_v3.2$ T-type Ca^{2+} channels)	Development of anxiety-related behavior	[5]
8	NA	Mice	Ablation of $\text{Ca}_v3.2$ T-type Ca^{2+} channels	Development of anxiety, sleep and memory disorders	[22]

Knockout of T-type Ca^{2+} Channels induces anxiety-related behavior

In contrast to the above-described studies, there have been studies reporting that the blockade or knockout of T-type Ca^{2+} channels induces anxiety. Gangarossa et al. described the role of T-type Ca^{2+} channels in the development of anxious behavior, memory formation and response to psychostimulants using $\text{Ca}_v3.2$ -deficient mice. The mice with genetic ablation of *cacna1h* gene, responsible for encoding $\text{Ca}_v3.2$ channels, showed an increase

in anxiety-related behavior in comparison with wild type mice assessed in different tests including light/dark conflict, open field, marble burying and elevated plus maze tests. These mice also exhibited disruption of hippocampus-dependent memory functions as assessed on Y-maze, novel object recognition and spatial object recognition tests. Moreover, such mice also exhibited decreased sensitivity to psychostimulants including cocaine hydrochloride (15 mg/kg, i.p.) and D-amphetamine sulfate (2 mg/kg, i.p.), which selectively increased the locomotor activity in wild-type mice, but not in $Ca_v3.2$ -deficient mice. Furthermore, administration of selective T-type calcium blocker TTA-A2 (1 mg/kg, p.o.) attenuated the cocaine- and amphetamine-induced hyperlocomotion in wild-type mice, suggesting that the actions of psychostimulants are mediated through the activation of the T-type calcium channels. Accordingly, it has been proposed that knockout of T-type calcium channels may lead to the development of anxiety-like behavior, memory disruption and induction of drug-induced behavioral alterations [5]. Another study has reported that ablation of genes responsible for expression of $Ca_v3.2$ or disruption of T-type channels within the CNS, particularly in the hippocampus, contributes to the development of anxiety, sleep and memory disorders in mice. The importance of $Ca_v3.2$ T-type voltage-gated channels specifically in the hippocampus was assessed using microarray transcriptome data of murine hippocampal RNA probes [22].

The study of Gironell and Lahoz described the role of ethosuximide in the development of anxious disorders during the exploration of this drug in subjects with essential tremor in an open label trial. The authors planned to conduct the clinical trial with 15 patients using a dose of 500 mg of ethosuximide daily (b.i.d.), but the trial was stopped after seven patients' participation because of lack of efficacy. Moreover, ethosuximide led to high incidences of adverse effects such as anxiety, nervousness and dizziness, causing the patients (in 80% of patients) to stop the trial [35]. It suggests that the blockade of T-type calcium channels induces the development of anxiety (Table 1).

Discussion

Anxiety disorders are one of the most common types of psychiatric disorders, and the modulation of T-type calcium channels is reported to modulate anxiety-related behavior in preclinical studies as well as in clinical studies [19], [32], [35]. Studies have shown that the blockade of T-type Ca^{2+} channels by the use of either pharmacological agents such as ethosuximide [33], endogenous peptide CRF [21] or plant extracts such as linalool or rosemary ameliorates anxiety and related behaviors [7], [19]. The overexpression of T-type calcium channels in the GAERS strain has been correlated with the development of anxiety [20]. Moreover, the blockade of T-type calcium channels with ethosuximide is shown to ameliorate anxiety, as an important comorbidity in epilepsy [32]. However, studies based on the knockout of the gene responsible for the expression of T-type calcium channels have shown that the studied rodents are more prone to anxiety-related behavior [5].

Accordingly, it is proposed that T-type calcium channels may have a dual role in anxiety. The blockade of T-type calcium channels with a pharmacological agent may possibly attenuate anxiety; however, knock-out of these channels may predispose to anxiety-related disorder. The precise mechanisms describing the duality of T-type calcium channels in anxiety are not explored yet. It may be hypothesized that normally functional T-type Ca^{2+} channel, i.e. at the basal state, may inhibit the development of anxiety. This convention is based on the T-type calcium channel knockout studies [5]. Conversely, under a given stressful stimulus, these T-type calcium channels may be upregulated, and thus, overactivation of these channels may lead to the development of anxiety-related behavior. The studies showing amelioration of anxiety in the presence of T-type calcium channel blockers in different models of anxiety support the above convention [7], [19]. The dual role of T-type calcium channels is also reported in the study of Ji et al., describing that ethosuximide ameliorates anxiety in low doses, while it produces anxiety at high doses in the larvae of zebra fish [31]. It again suggests that inhibition of T-type calcium channels to a certain level may attenuate anxiety. However, the complete blockade of these channels or knockout of these channels may produce anxiety.

The molecular mechanisms involved in T-type calcium channel-mediated modulation of anxiety-like behavior are not explored yet. It has been observed that the modulation of T-type Ca^{2+} channels leads to the alterations in the level of various neurotransmitters including dopamine, serotonin and glutamate [36], [37], [38], [39]. The modulation of these neurotransmitters, i.e. decrease in the levels of dopamine and serotonin along with an increase in the level of glutamate, is associated with the development of anxiety-related disorders [40], [41], [42], [43]. Therefore, it may be proposed that the T-type Ca^{2+} channel may play an important role in modulating anxiety-related disorders by alterations in the levels of dopamine, serotonin and glutamate via unexplored signaling pathways. Moreover, some studies have shown the possible involvement of protein kinase C pathway in inhibiting T-type calcium channels [30]; however, their precise association in anxiety-related behavior is unex-

plored. Accordingly, future studies shall be designed to fully elucidate the dual role of T-type calcium channels along with the molecular mechanisms/signaling pathways contributing in modulating anxiety behavior.

Conclusions

T-type calcium channels may serve as an important target in anxiety-related disorder. These channels may inhibit anxiety in normal or basal state. However, the overactivation of channels during a stressful condition may produce anxiety, and hence, blockade of these channels during a stressful state may possibly ameliorate anxiety.

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