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ISSN 2177-7853

Experimental emotional disorders in epilepsy and the implication of muscarinic acetylcholine receptors: a forgotten putative therapeutic target

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Histórico do Artigo
Recebido em: 28/09/2016
Aceito em: 15/11/2016

Keywords:
Anxiety
Epilepsy
Acetylcholine
Muscarinic receptors.

ABSTRACT

Over the last decades it has been observed a major breakthrough in the development of new drugs for the treatment of emotional disorders. Regardless of this evolutionary framework, the neurobiological mechanisms involved in anxiety process are complex and new approaches in the treatment of these disorders are still required, taking into account the resistance to therapies and therapeutic side effects. Despite the clear involvement of muscarinic acetylcholine receptors (mAChRs) in the modulation of many physiological functions, including synaptic transmission, epileptogenesis and emotional states, little attention has been paid to this neurotransmitter system and its feasible action in the treatment of psychiatric diseases. In this sense, the present review aims to highlight the involvement of the mAChRs in the regulation of emotional disorders in healthy and epileptic subjects or animals. Recent findings have revealed the modulatory role of hippocampal circuitry in the regulation of experimental anxiety following the activation of cholinergic afferents in limbic structures. Such investigations may provide a rational basis and encourage the development of novel drugs targeted at mAChRs. Considering the limitations in the translational field of mental disorders, the lack of mAChR subtype-selective ligands and the broad distribution of multiple mAChR in the central nervous system, the information discussed here demonstrate an alternative strategy for the investigation of underlying mechanisms of long-term anxiety besides assisting in the refinement of preclinical research related to these disorders.

Palavras-chave
Ansiedade
Epilepsia
Acetilcolina
Receptores muscarínicos

Transtornos emocionais na epilepsia experimental e o envolvimento de receptores colinérgicos muscarínicos: um potencial alvo terapêutico esquecido

RESUMO - Nos últimos anos tem-se observado um grande avanço no desenvolvimento de novas drogas para o tratamento dos diferentes transtornos emocionais. Apesar deste quadro evolutivo, os mecanismos neurobiológicos envolvidos nos processos de ansiedade são complexos e novas abordagens para o tratamento desses transtornos ainda são necessários, levando em consideração a resiliência a algumas terapias e os efeitos colaterais proporcionados por estas. Mesmo com a evidente participação dos receptores colinérgicos muscarínicos (mAChRs) na modulação de inúmeras funções fisiológicas, incluindo a

transmissão sináptica, epileptogênese e estados emocionais, pouca atenção tem sido voltada a este sistema de neurotransmissão e sua potencial ação terapêutica. Nesse sentido, a presente revisão tem como objetivo ressaltar o envolvimento dos mAChRs na regulação de transtornos emocionais em sujeitos e animais saudáveis e epilépticos. Achados recentes revelaram o papel modulatório da circuitaria hipocampal na regulação da ansiedade experimental após a ativação de aferentes colinérgicos de estruturas límbicas. Tais estudos podem fornecer uma base racional e encorajar o desenvolvimento de novas drogas que atuem sobre os mAChRs. Levando em consideração as limitações translacionais no campo dos transtornos psiquiátricos, a ausência de ligantes seletivos para os subtipos de mAChRs e ampla distribuição de múltiplos mAChRs no sistema nervoso central, as informações discutidas aqui demonstram uma alternativa para investigação dos mecanismos subjacentes à ansiedade de longo prazo além de auxiliar no refinamento de pesquisas pré-clínicas relacionadas a estes transtornos.

1. Introduction

The term "comorbidity" is known in clinical practice by the coexistence of various disorders that affect the patient at the same time (1). Several studies have shown that the incidence of emotional disorders is considerably higher in epileptic subjects compared to other chronic diseases (2-4) whereas depression and anxiety disorders have affected epileptic humans and animals in a similar manner (5-9).

The neurobiological mechanisms involved in these comorbidities (i.e., anxiety and epilepsy) are poorly understood and systematic studies in this area are scarce (Figure 1). Despite the lack of evidence, most researchers have focused their investigation on the behavioral analysis of kindled epileptic rodents (10-12) or by pharmacological induction of seizures (13, 14), reporting therefore the anxiogenic-like profile of them as the result of ictal discharges and/or convulsive behaviors.

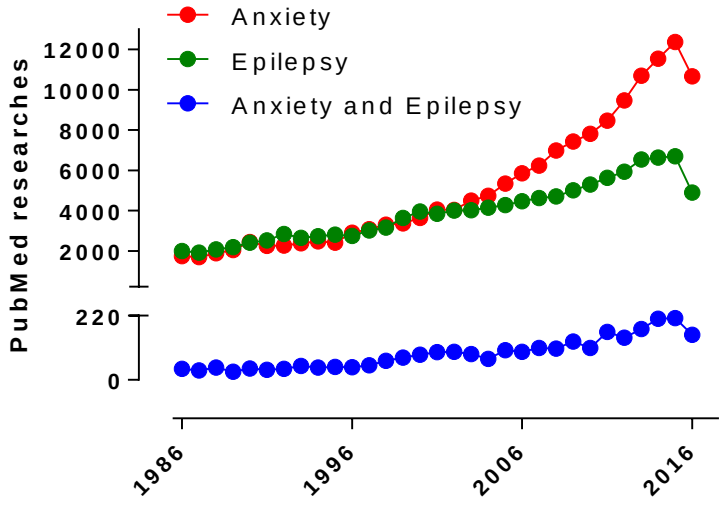

Figure 1. The annual number of papers published (during the last 30 years) in different journals using the search terms "anxiety", "epilepsy" and "anxiety and epilepsy", available at the PubMed base, developed by the National Center for Biotechnology Information (NCBI) and maintained by the National Library of Medicine of the United States (adapted from www.pubmed.com, accessed on Sep, 22nd 2016).

2. Material and methods

The present study is characterized by a systematic review of advanced literature carried out by the consultation of scientific and medical researches available at PubMed, Google Scholar and Science direct database. The search of manuscripts was done mainly using the headings “anxiety”, “epilepsy”, “comorbidities” and “acetylcholine receptors”. Thus, investigations comprising the topics addressed here (i.e., experimental studies of emotional and epileptic disorders involved with the muscarinic cholinergic system) were included in the manuscript.

3. Results

The ability to reproduce human diseases in animal models is a great benefit for modern experimental medicine (15). The animal model of pilocarpine - a muscarinic nonselective cholinergic agonist - presents an isomorphic high degree with temporal lobe epilepsy (TLE) in humans and it has been used by different research groups since its first report 35 years ago (16-18). This model presents several advantages, including the rapid induction of status epilepticus (SE); presence of a latent period followed by the appearance of spontaneous recurrent seizures (19, 20), promotion of scattered lesions in the brain which are usually located in the same brain regions affected in humans and associated with the reorganization of the neural network in hippocampal regions (21).

In animal models, the systemic injection of high doses of pilocarpine (over 300 mg/kg) promotes behavioral epileptogenic episodes associated with electrographic discharges that progressively increase in duration and dispersion (20). Following the induction and maintenance of the SE, the administration of atropine (a cholinergic receptor antagonist) is no longer able to control seizures, suggesting the participation of non-cholinergic elements (e.g., glutamate, GABA and dopamine) in the continuation of these seizures. In particular, the involvement of glutamatergic NMDA-type receptors (NMDARs) in this process may promote anticonvulsant effects when are blocked or antagonized (22-26). For instance, epileptic mice show increased anxious-like responses when evaluated long-after pilocarpine treatment, as observed in different behavioral tests (e.g., open field, light-dark box, elevated plus-maze, hole-board tests), besides an impairment in spatial learning and memory when assessed in the Morris water-maze test and hippocampal lesions (mainly in pyramidal cells of CA1 and CA3 regions), reflecting many cognitive behavioral responses also observed in epileptic humans (27-29).

Acetylcholine exerts its physiological functions binding to nicotinic (ionotropic) or muscarinic (metabotropic) receptors (mAChRs), where they regulate neuronal excitability, synaptic transmission and plasticity (30, 31). Notably, mAChRs play pivotal functions in the hippocampus (e.g. learning and memory formation) by depolarizing pyramidal neurons and facilitating the induction of long-term potentiation (LTP) (32, 33). These receptors are further divided into two classes according to the functionality of the G protein-coupled receptors, modulating the activity of a wide range of phospholipases, ion channels, protein kinases and other signaling molecules (34). Receptors subtypes M1, M3 and M5 are selectively coupled to G proteins from the Gq/G11 family, while M2 and M4 receptors preferentially activate G proteins of the Gi/Go family (for review see 35).

Cholinergic neurons are sparsely distributed throughout the central nervous system, located in the brainstem, projecting caudally to the spinal cord and midbrain tegmentum, the diencephalon and rostral to the telencephalon (36). The cholinergic septo-hippocampal pathway is closely involved in the regulation of hormones and behavioral stress responses in rodents (37-41). The administration of anticholinergic drugs and the injury of ascending

cholinergic projections promotes neural and behavioral effects similar to those found after experimental manipulation on the noradrenergic and serotonergic systems (besides similarly projecting their axons to those systems), modifying the neural signaling in neural targets involved in fear and anxiety states, such as the hippocampus and amygdala (42).

Wall and colleagues (43) showed that an increased cholinergic transmission in the limbic region below the medial prefrontal cortex induces anxiety-like responses and improves working memory in mice, as also reported by De Mello and colleagues (44) in rats. Furthermore, the injection of pirenzepine (an antagonist of the M1-subtype mAChRs) produce anxiolytic-like responses, whereas McN-A-343 (an agonist of the M1-subtype muscarinic receptor) promotes anxiogenic-like responses, suggesting that cholinergic activity in the medial prefrontal cortex and forebrain (especially through the mediation of M1-subtype receptors) can exert significant roles in the regulation of anxiety (43). In contrast, studies conducted in healthy and geriatric patients diagnosed with depression show the presence of anxious responses after the administration of scopolamine - an antagonist of muscarinic receptors (45, 46). Similarly, the central or systemic administration of scopolamine in rats produced anxiogenic-like effects in the light-dark box test (47, 48), whereas the blockade of M1-subtype receptors - but not the M2-subtype receptor - increases anxiogenic-like behaviors in rats evaluated in the social interaction test (49). In contrast, a recent study demonstrates opposite effects when a non-selective or M1- and M4-subtype selective agonists are infused into the insular cortex, showing that the activation of mAChRs can produce anxiolytic effects while their inhibition increases anxiety (50). In this sense, cholinergic effects on experimental anxiety appear to be via- and locally-dependent, emphasizing a complex and poorly understood mechanism.

Moreover, activation of M1-subtype receptors potentiates excitatory transmission of hippocampal pyramidal cells via NMDARs (51). Particularly, NMDARs are characterized as glutamate-gated cation channels with high calcium permeability and exert pivotal function in higher organisms maintenance (52). Further, these receptors require the binding of two co-agonists to be activated, glycine and L-glutamate (53). They are composed of different subunits: NR1 (by binding to the glycine) presenting excitatory regulation, NR2A-D (by binding to the glutamate) and NR3A or B (binding to glycine) presenting inhibitory regulation (54).

Grishin and colleagues have shown that the stimulation of M1 mAChRs reduces the ionic currents of NMDARs located in the pyramidal cells of the CA3 region (55, 56). Further, blocking the functioning of G proteins or depleting intracellular stores of Ca2+ prevented muscarinic depression of ionic currents from NMDARs, indicating that the pathway involved with the M1-subtype receptor, the activation of G protein and the Ca2+ release are the initial steps in the transduction mechanism. Thereafter, the activation of Ca2+-dependent/calmodulin, which in turn activates the protein tyrosine phosphatase, may lead to desensitization (down-regulation) of NMDARs (56). Accordingly, changes in the cholinergic functioning are also linked to emotional disorders such as anxiety (57, 58) and cognitive alterations, interfering with the processes of learning and memory (59), attention (60), vigilance (57) and epilepsy (61).

The activation of mAChRs (especially the M1-subtype) can potentiate excitatory transmission in the central nervous system through the activation of NMDA-type receptors (51). The glutamatergic system is known to act effectively in the modulation of anxiety and fear status. The blocking of excitatory activity generated by ionotropic receptors (e.g. antagonists of the NMDARs) can promote anxiolytic responses in animals tested in different unconditioned test of anxiety (54). The anxiolytic-like effects elicited by NMDAR antagonists appear to reflect the blockade of these receptors in the hippocampus, considering that mice that do not express the NR1 subunit of the NMDAR in the granule

cells of the dentate gyrus exhibit a normal LTP in the CA1 area, although presenting an anxiolytic-like profile in anxiety tests (62). Moreover, mice that do not express the NR2B subunit of the NMDAR in the pyramidal and granular hippocampal cells also exhibit anxiolytic-like responses (63).

The upregulation or downregulation of NMDARs is implicated in neurological disorders such as schizophrenia (64), mood disorders (65) and anxiety (54). As reported, numerous transduction pathways converge towards the NMDARs modulating its gain and hence the efficiency of synaptic transmission (66). The administration of a non-convulsive dose of pilocarpine may also alter the expression of hippocampal NMDARs in the rat (67), suggesting that the activation of mAChRs can modify anxiety through hippocampal plastic changes and cell excitability.

Moreover, dysfunctions of the cholinergic system may induce stress states (68) by initiation of long-term changes in cholinergic gene expression, both in the neocortex and hippocampus of mice (39). The stress responses are activated or facilitated by neurons in the brainstem (including the cholinergic system) that project to the paraventricular nucleus (PVN) of the hypothalamus and activate the hypothalamic-pituitary-adrenal axis (HPA) (69, 70). In mammals, physiological responses (e.g., tremors, muscle tension, sweating, palpitations, dizziness) triggered by the presence of a stressful stimulus are mediated primarily by the HPA axis (71, 72). Neural signals associated with the stimuli are characterized by hypothalamic-regulated endocrine responses, especially the PVN region - an integrative center that receives and coordinates neuroendocrine, autonomic, cognitive and emotional information and responsible for glucocorticoids release initiation (73). Both corticotrophin releasing hormone (CRH) and arginine vasopressin are secreted by the PVN in the hypophyseal portal system, where they reach the anterior pituitary gland and synergistically stimulate the release of adrenocorticotropic hormone (ACTH), which is transported through the bloodstream to the adrenal cortex, where it stimulates the release of glucocorticoids (74).

Several studies have mentioned the hippocampus as responsible to exert an inhibitory function on the HPA axis functioning, since their stimulation decreases the secretion of glucocorticoids in both rats and humans (75, 76), besides being involved in finalizing these responses after a stressful situation (77, 78). The levels of acetylcholine in the hippocampus and cortex may increase considerably after restraint stress in rats (79). The way in which cholinergic function converges to regulate hippocampal efferent projections in response to stress is still unknown, although it is suggested that the effects elicited by the activation of muscarinic and nicotinic receptors on excitatory and inhibitory transmission serve to regulate the theta activity (80, 81), a critical tool in the processing of memory, mood disorders and anxiety (82).

The theta activity in the hippocampal formation - a pattern neuronal firing in the with slow EEG activity (5-10 Hz) - is the result of phasic activity of subcortical systems mainly modulated by cholinergic and serotonergic projections (42). Several researchers have linked the activation of mAChRs with the modulation of theta rhythm in limbic structures that receive cholinergic afferents (83). Gray and McNaughton proposed that theta rhythm in the septo-hippocampal system is involved in the modulation of anxiety states, since lesions of this system or the administration of anxiolytic drugs in this area inhibits hippocampal theta activity in rats (42, 84). The treatment with pilocarpine is able to increase the incidence of hippocampal theta frequency long after (85, 86). An increased temporal synchronization of this rhythm is also observed in patients with TLE (87) and related to fear and anxiety responses (88, 89), pointing to an important role of theta frequency in the modulation of these comorbidities. Information about the neuronal EEG pattern implicated in the modulation of anxiety states can better denote the role of the

cholinergic system in the control of emotional responses observed in rodents, since the hippocampal theta rhythm act as a "pacemaker" in the septo-hippocampal system controlling anxiety (42). In this sense, temporary changes in hippocampal rhythm triggered by pilocarpine may be involved in anxiogenic responses, resembling those observed in humans who report "anxiety-like feelings" during a "threat" and, thereafter, present an increased hippocampal theta activity during this stimulus (90).

Over the last decades it has been observed a major breakthrough in the development of new drugs for treatment of emotional disorders (91). Regardless of this evolutionary framework, new approaches are still required taking into account the resistance of some diseases to therapies currently employed and their side-effects (92). Even though mAChR modulators are currently used in clinical treatment for glaucoma, gastrointestinal and urinary disorders, asthma, ulcer, certain forms of cardiac arrhythmias, movement disorders and Parkinson´s disease (93), its use is many times limited due to side effects caused by the non-selective activation or blockade of all or various mAChRs (34). A pilot study conducted by our research group showed that rats that did not develop SE after the systemic injection of a high dose of pilocarpine (350 mg/kg) present an anxiogenic-like profile when evaluated in the elevated plus-maze test one month after treatment (85). These results aroused great interest in order to better understand these effects, since much of the current researches have focused its attention in the generation and propagation of seizures induced by convulsant agents, minimizing or ignoring the consequent emotional or cognitive outcomes caused by the treatment. Moreover, most of current preclinical studies denote a significant number of limitations encountered in the translational field of animal models for psychiatric disorders, with a low rate of clinical implementation of alternative therapies that could bring light to treatments for epilepsy and anxiety, becoming necessary the search for new strategies with different therapeutic targets.

The long-term anxiogenic-like effects induced by a non-convulsive dose of pilocarpine were later observed to be critically mediated by prosencephalic connections, once the temporary inactivation of the fimbria-fornix and post-commissural fornix pathways - the main hippocampal pathways to the septal and diencephalic areas (94) - attenuates the anxiogenic-like responses of animals evaluated one month after injection of pilocarpine in the elevated plus-maze test (95). Anxiogenic-like responses were also observed in rats evaluated in different tests of anxiety such as the T-maze and open field apparatus long after pilocarpine treatment (85).

Even though the injection of a high dose of pilocarpine (i.e. 350 mg/kg) may not to promote SE or recurrent spontaneous seizures in 35 % of treated animals - a phenomenon previously reported (44, 96) - electrographic seizures during EEG recordings comprising high incidence of spike-wave discharges are clearly observed up to one month after the treatment, followed by reduced L-[3H]-glutamate uptake and cell viability in the hippocampal (85), evidencing that anxiogenic-like effects observed in these animals could result not just from seizures but also from hippocampal lesions, in disagreement with animals that also showed anxiogenic-like responses after treatment with a lower dose of pilocarpine (150 mg/kg) and did not develop any epileptogenic characteristics. These results show that the activation of mAChRs by pilocarpine may promote effective long-term changes in neuronal circuitry of rats, regardless of electrographic seizures activity, suggesting this preparation as of great potential in the research of experimental long-term anxiety.

According with Laborit (97), anxiety becomes evident when an adaptive response is not feasible before a potentially aversive stimulus, depending on the activation of the HPA axis. The anatomical alterations produced by stress in the hippocampus and amygdala (98) seem consistent with their different roles in the circuitry: the hippocampus has an

important inhibitory function of the HPA axis whereas the amygdala shows excitatory actions (99). As previously reported, the release of adrenal steroid hormones (glucocorticoids) is a normal response to stress, although when chronically occurring, is associated with various diseases and neurotoxic events such as the excessive glutamate release in the hippocampus (100). Interestingly, long-term exposure to glucocorticoid also decreases seizure thresholds in different animal models of TLE (101-103), although the mechanisms underlying these effects on neuronal excitability and seizures are poorly understood and limited. Further, overactivation of the HPA axis is consistely found in subjects with TLE and linked to epilepsy symptoms and psychopathologies (104).

Children evaluated during the first seizure reported higher anxiety feelings compared with healthy subjects and 45 % of psychiatric commorbidities were diagnosed before recognition of the first seizure (105, 106). In epileptic rodents, increased levels of corticosterone and CRH-positive neurons in the hypothalamus are observed following the SE (107, 108). Thus, the relationship between emotional disorders and epilepsy seems to be bidirectional once, not just patients with epilepsy are more likely to be anxious when compared with healthy subjects, as a high risk of diagnosed anxiety years before (and after) epilepsy occurrence is observed (109), denoting a shared etiological mechanism underlying these pathologies.

Recent studies in rodents shown the long-term effects caused by a single exposure to a stressor stimulus (e.g., shock, restraint) resulting in desensitization of glucocorticoid receptors and dramatic increase in plasma levels of corticosterone and ACTH (in a lesser extent), suggesting the existence of specific influences of the stressor stimulus in the regulation of adrenocortical secretion, independent of ACTH (110). Rats treated with an anxiogenic dose of pilocarpine present long-term elevated plasmatic levels of stress markers such as corticosterone and ACTH associated with a decreased expression of hippocampal glucocorticoid receptors, suggesting the mediation of central pathways and epigenetic changes activated by the cholinergic stimulation (67).

The participation of hippocampal modulators in experimental anxiety following mAChR activation highlights the important role of this alternative target in the treatment of anxiety states. Although systematic studies regarding the intracellular events implicated in the modulation of mAChRs over glucocorticoids and NMDARs need to be further investigated, these recent findings highlight the muscarinic-NMDARs crosstalk and pivotal interference in the regulation of the HPA axis, which may explain the anxiogenic effects observed in epileptic and non-epileptic rats treated with pilocarpine.

4. Conclusion

In summary, the present review aims to highlight the involvement of the muscarinic cholinergic system in the regulation of emotional disorders in healthy and epileptic subjects and animals. Important to note, recent findings regarding the modulatory role of hippocampal circuitry in the regulation of experimental anxiety following activation of cholinergic afferents in limbic structures was brought to light, providing a rational basis and encourage the development of novel drugs targeted at mAChRs. Taking into account the limitations in the translational field of psychiatric disorders, the lack of mAChR subtype-selective ligands currently available and the broad distribution of multiple mAChR in the CNS, the information discussed here demonstrate alternative strategies for the investigation of long-term anxiety besides assisting in the refinement of preclinical research related to these disorders.

Acknowledgement: This work was supported by CNPq, which provided a research grant to TCM de Lima and CAPES/PNPD, National Institute of Translational Neuroscience (INNT) and NENASC project (PRONEX Program CNPq/FAPESC) which provided research grants to AA Hoeller, CR de Carvalho and R Walz.

Conflict of interest: The authors declare that there is no conflict of interests regarding the publication of this article.

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