Clinical and Experimental Health Sciences

Review / *Derleme*

Muscarinic M1 and M2 receptors, fasting and seizure development in animals

M1 ve M2 muskarinik reseptörler, açlık ve hayvanlarda nöbet gelişimi

Merve Saygı Bacanak[1,2]

[1] Department of Medical Pharmacology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey
[2] First and Emergency Aid Program, Department of Medical Services and Techniques, Vocational School of Health Services, Marmara University, Istanbul, Turkey

ORCID IDw of the authors: M.S.B. 0000-0001-9774-2657

Cite this article as: Bacanak M. Muscarinic M1 and M2 receptors, fasting and seizure development in animals. Exp Health Sci 2018; 8: 308-313.

ABSTRACT

Muscarinic M_1 and M_2 receptors are widely distributed in the brain and contribute to various physiological and pathological functions. It is well known that enhancement of cholinergic activity produces convulsions in animals. Pilocarpine, the M_1 muscarinic receptor agonist, is commonly used to induce seizures in rodents. On the other hand it has been reported that fasted rats and mice pretreated with the M_1 and M_2 receptor antagonist atropine, scopolamine or biperiden develop convulsions after food intake indicating cholinergic hypoactivity as the underlying mechanism. This review will present pharmacological data for the M_1 and M_2 receptors in the brain and focus on the contrasting findings related to their contribution to convulsions.

Keywords: Seizures, receptors, muscarinic m1, muscarinic m2

ÖZ

M_1 ve M_2 muskarinik reseptörler beyinde oldukça yaygındır ve çeşitli fizyolojik ve patolojik fonksiyonlara katkıda bulunurlar. Kolinerjik aktivitenin arttırılmasının hayvanlarda konvülsiyon oluşturduğu iyi bilinmektedir. M_1 muskarinik reseptör agonisti olan pilokarpin ise kemiricilerde nöbet oluşturmak için sıklıkla kullanılmaktadır. Öte yandan M_1 ve M_2 muskarinik reseptör antagonisti atropin, skopolamin veya biperiden uygulanan aç fare ve sıçanlara yem verildikten sonra konvülsiyon oluştuğu ve bunun altında yatan mekanizmanın kolinerjik hipoaktiviteyi gösterdiği bildirilmiştir. Bu derlemede beyindeki M_1 ve M_2 reseptörlere ilişkin farmakolojik bilgiler sunulacak ve bu reseptörlerin konvülsiyonlara katkılarına ilişkin çelişkili bulgular üzerinde durulacaktır.

Anahtar Kelimeler: Nöbetler, reseptörler, m1 muskarinik, m2 muskarinik

INTRODUCTION

Subtypes, regional distribution and synaptic localization of muscarinic receptors

Five muscarinic receptor subtypes, M_1, M_2, M_3, M_4 and M_5 have been identified. Their regional distribution and function in the brain are shown in Table 1. Muscarinic receptors in brain are located in neurons and glia cells. The most prevalent subtypes in rat brain are M_1 and M_2 receptors (1).

M_1, M_3 and M_5 receptors preferentially couple to Gαq subunit that activate phospholipase C and generate second messengers, inositol triphosphate (IP3) and diacyl glycerol (DAG) and so intracellular calcium levels increase (Figure 1). These receptors may also activate phospholipase A_2 and phospholipase D in certain cells (2). On the other hand muscarinic M_2 and M_4 receptors preferentially couple to Gαi/o subunit that inhibits adenylate cyclase and reduce the production of second messenger cyclic adenosine monophosphate (cAMP).

In brain, M_1 receptors are most commonly located postsynaptically while M_2 receptors are most commonly located presynaptically (3) (Figure 1). Blockade of postsynaptic muscarinic receptors reduces the effects of acetylcholine, whereas blockade of presynaptic muscarinic autoreceptors causes an increase in acetylcholine release (4). M_2 receptor was shown to be the main presynaptic autoreceptor in hippocampus and cerebral cortex (4), while both M_1 and M_2 receptors are located presynaptically and postsynaptically in cerebral cortex and hippocampus (3, 4, 5). Blockade of the M_1 receptors increases acetylcholine release in rat cerebral cortex and hippocampus (5, 6), whereas does not change acetylcholine release in human cerebral cortex cell culture (7). Presynaptic muscarinic autoreceptors (M_1, M_2, M_3, M_4) inhibit acetylcholine release and are also located on noncholinergic nerve terminals as heteroreceptors and contribute to the effects of acetylcholine (8, 9). Blockade of M_1 heteroreceptors leads to a decrease in dopamine release (10) and blockade of M_2 heteroreceptors leads to an increase in glutamate release (8).

Correspondence Author/Sorumlu Yazar: Merve Saygı Bacanak **E-mail/E-posta:** saygimerve@hotmail.com

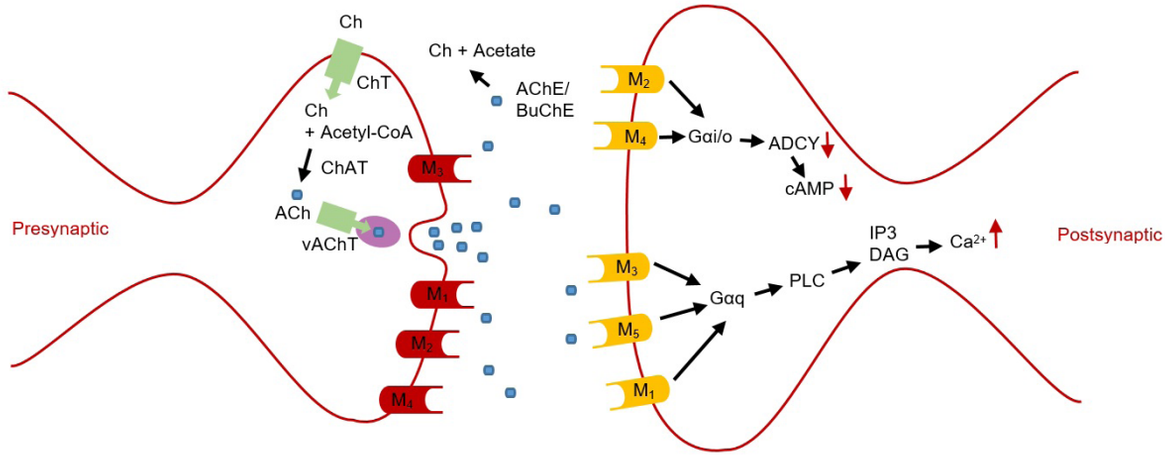

Figure 1: Cholinergic synapse, muscarinic receptors and synaptic neurotransmission (50)

Abbreviations: ChT, high-affinity choline transporters; ChAT, choline acetyltransferase; vAChT, vesicular ACh transporter; AC, acetylcholine; AChE, acetylcholinesterase; BuChE, butyrylcholinesterase

Table 1: Distribution and function of muscarinic receptor subtypes

Muscarinic receptor subtype	Widely distributed region in the brain	Associated physiological functions
M_1	Cerebral cortex, hippocampus, striatum (41)	Receptor stimulation → Regulation of learning and memory like cognitive functions (18), regulation of locomotor activity (42), increases wakefulness while reducing delta sleep (43)
M_2	Forebrain, thalamus, motor neurons (1)	Receptor blockade à Increased cognitive performance including memory (21), decreased REM sleep-like state (44) Receptor stimulation → antinociceptive effect (45)
M_3	Hypothalamus, hippocampus (21)	Receptor knockout → Reduced food intake and increased locomotor activity, decreased pituitary and serum growth hormone (GH) and prolactin (46), Receptor stimulation → regulation of metabolic functions and longitudinal growth (46), regulation of learning and memory like cognitive functions (47)
M_4	Corpus striatum (21)*	Receptor knockout → Increased locomotor activity and dopaminergic activity so contribution to antiparkinsonian effect (21) Receptor stimulation → Contribution to antipsychotic effect (21), antinociceptive effect (45)
M_5	Substantia nigra pars compacta, ventral tegmental area (21)**	Receptor knockout → Abolished cholinergically induced cerebral vasodilation (48) Receptor stimulation → Increased dopamine release in substantia nigra pars compacta, inhibition of dopamine release in striatum (49)

*M_4 receptors are distributed in the corpus striatum being co-localized with dopamine receptors (21).
**M_5 is the only muscarinic subtype expressed by the dopamine-containing neurons of the substantia nigra pars compacta (21).

Muscarinic agonists and antagonists

Muscarine, pilocarpine and arecoline are the naturally occurring muscarinic agonists. Methacholine, carbachol, bethanechol and oxotremorine are the most known synthetic muscarinic agonists. Oxotremorine-M is the most potent N-methyl quaternary derivative of oxotremorine and cannot pass through the blood brain barrier. Arecoline and pilocarpine are the partial agonists. Selectivity and affinity for the muscarinic receptor subtypes differ between the muscarinic agonists and antagonists. Pilocarpine demonstrated selectivity for M_1 and M_3 subtypes regarding the intrinsic relative activity (RA_i) (11). In this study arecoline, carbachol and oxotremorine-M lacked marked selectivity among M_1 to M_4 receptors. Xanomeline, the synthetic muscarinic agonist demonstrated functional selectivity for the M_1 and M_4 (12) muscarinic receptor subtypes. However binding studies showed similar affinity of xanomeline at all five subtypes (13).

The affinities of atropine, scopolamine, biperiden and pirenzepine, the main muscarinic receptor antagonists are shown in Table 2. Atropine, the nonselective muscarinic receptor antagonist has equal affinity for all muscarinic receptor subtypes, however scopolamine has lower affinity for M_2 receptors than the other subtypes. Biperiden has highest affinity for M_1 receptors. And pirenzepine is the selective antagonist of M_1 subtype. According to the in vitro muscarinic receptor radio ligand binding assays, the affinities of pirenzepine, biperiden, scopolamine (14) and atropine (15) were 98, 46, 6 and 2 fold higher for M_1 subtype than M_2 subtype (Table 2). Additionally atropine and scopolamine had showed 10 times higher affinity for presynaptic receptors than postsynaptic receptors (16).

Table 2: Comparison of binding affinities for atropine, scopolamine, biperiden and pirenzepine at human muscarinic receptors as Ki values

	M_1	M_2	M_3	M_4	M_5
Atropine	0,17	0,339	0,209	0,107	0,316
Scopolamine	0,83 (0,05)	5,3 (1,4)	0,34 (0,06)	0,38 (0,07)	0,34 (0,11)
Biperiden	2,2 (0,23)	102 (24)	5,3 (1,3)	3,1 (0,8)	4,4 (1,4)
Pirenzepine	43 (14)	4200 (1370)	468 (172)	148 (53)	237 (122)

Human cloned receptors were expressed in Chinese hamster ovary cell membranes. Binding was measured as competition with [3H]N-methyl-scopolamine. Data represent as Ki (mean ± S.E.M.) in nanomolar (14, 15).

CLINICAL AND RESEARCH CONSEQUENCES

Roles of musarinic receptors in physiological functions and pathological processes

As shown in Table 1, muscarinic receptors participate in many physiological functions including learning and memory formations, locomotor activity, sleep-wake cycle, regulation of heart rate, growth hormone, prolactin, gastric acid and salivary secretions and contraction of smooth muscles.

Muscarinic receptors contribute to the pathophysiology of various neurological disorders. It is known that cognitive function is impaired in Alzheimer's disease and schizophrenia. Selective M_1 agonism has been shown to retard the age, Alzheimer's disease or schizophrenia related dementia and cognitive deficits with few side effects (17). Additionally M_1 receptors have been demonstrated to control amyloid precursor protein (APP) processing and the generation of the neurotoxic APP fragment, amyloid β-peptide (Aβ) in Alzheimer's disease (18). Various researchers have suggested that cholinergic function may be improved by selective blockade of M_2 receptors alone or together with M_1 agonism in early stages of Alzheimer's disease. Post-mortem and brain-imaging studies have shown that M_1 muscarinic receptor protein and M_1 receptor mRNA were reduced in different brain regions of patients with schizophrenia (19). Benztropine, a selective M_1 receptor antagonist, reduces the adverse side effects of antipsychotic treatments in schizophrenic patients (20). However M_1 agonists are promising for reversing some of the cognitive impairments associated with schizophrenia (19). So the efficient way for the treatment of schizophrenia regarding the M_1 muscarinic receptor agonism vs antagonism remains unclear. In the caudate putamen, muscarinic M_2 receptors act as inhibitory heteroreceptors on dopaminergic terminals. Therefore, selective M_2 antagonism may provide beneficial effects in schizophrenia, where dopaminergic transmission is increased (21). Dopaminergic neurons in the striatum are lost in Parkinson's disease and this causes an imbalance between dopaminergic and cholinergic effects with an excess of cholinergic effects. This is associated with increased striatal acetylcholine levels, which contributes to the development of the motor signs typically associated to Parkinson's disease. Selective M_1 receptor antagonists are used for the prevention and the treatment of dyskinesia and the treatment of dystonia (20). Muscarinic M_4 receptor knockout mice displays enhanced locomotor activity and D_1 dopaminergic receptor related effects (21). M_4 muscarinic receptors seem to suppress D_1 receptor function. So, selective blockade of M_4 receptors are being investigated in the treatment of Parkinson's disease.

Convulsions induced by muscarinic agonists

Enhancement of cholinergic activity by M_1 and M_2 muscarinic receptor agonists carbachol (22) and pilocarpine (23) or the acetylcholinesterase inhibitor soman (24) produce convulsions in animals.

Pilocarpine, a nonspecific muscarinic receptor agonist is commonly used to induce seizures in mice and rats with high systemic or intracerebral administrations. Pilocarpine-treated animals demonstrate structural damages (23) and tonic-clonic generalized seizures (25) like in humans with temporal lobe epilepsy. Animals display status epilepticus (SE) followed by a latent period. After this seizure-free period, spontaneous recurrent seizures (SRSs) are generated (25). M_1 muscarinic receptor knockout pilocarpine treated animals display no seizure activity. So M_1 receptors seem to be responsible for the seizure development (26). After binding to M_1 receptors, phospholipase C is activated. DAG and IP3 are produced, Ca^{++} and K^+ currents are altered. Consequently, neuronal excitation is increased. The activity of ATPases in the hippocampus may be reduced. So, the plasma membrane could not be repolarized. As the Ca^{++} ions could not be extruded, the increased Ca^{++} levels elevates glutamate release and this leads to SE. The glutamate permits Na^+ and Ca^{++} influx, thus the Mg^{++} is moved out of the cell. Mg^{++} acts as a blocker on the N-methyl-D-aspartate (NMDA) receptors. When Mg^{++} is extruded, glutamate activates the NMDA receptors. This causes excessive Ca^{++} entry and subsequent excitation and death of the cell. On the other hand, M_2 receptor activation by pilocarpine inhibits adenylate cyclase. This causes a reduction in the acetylcholine release and the excitability of the brain (27).

Convulsions induced by muscarinic antagonists

Enginar et al. showed that mice deprived of food displayed seizures after food intake in a study exploring the effects of scopolamine on memory and learning processes (28). In a series of experiments, mice and rats fasted for ≤ 48 h and treated with antimuscarinics, scopolamine, atropine or biperiden developed convulsions soon

after finding and eating the food pellet (29, 30). Antimuscarinic pretreatment and access to food are required in the generation of convulsions. Hypoglycaemia was prevented by glucose intake but convulsion development not. So, the contribution of a hypoglycaemic effect during fasting was ruled out (29). The binding characteristics of glutamatergic receptors were changed after fasting for 48 h. These changes were moderately blocked by scopolamine pretreatment and eating food (31). The convulsions seem similar with a form of reflex seizures called eating epilepsy regarding the triggering factors, exhibition of the seizure activity and response to antiepileptics (32). This new method/technique may provide insight into the seizures in patients.

It is very interesting that scopolamine has actually anticonvulsive effect. Scopolamine prevents seizures induced by anticholinesterase soman (24) and muscarinic agonist pilocarpine (22). The convulsive effects of scopolamine, atropine and biperiden have been suggested to be an anticholinergic effect arising from the antagonism of postsynaptic M_1 and/or M_2 muscarinic receptors. These receptors show different postsynaptic and presynaptic localizations and distributions in the brain and have different autoreceptor and heteroreceptor characteristics as mentioned above. Which of these receptors is responsible for the convulsions has not been fully understood yet. It has been reported that the topical administration of high concentrations of antimuscarinics to the brain produced seizures (33, 34). This effect has been suggested to imply an anticholinergic as well as cholinergic activity due to their efficacy in increasing the release of acetylcholine.

Prolonged fasting has been shown to alter the expression or binding characteristics of various receptors in brain. For instance; fasting for 48 h produced changes in glutamatergic receptors (31) and 120 h food deprivation decreased the gamma aminobutyric acid (GABA) receptors in the cerebellum (35). In the studies investigating the receptor binding and gene expression changes in rats with insulin-induced hypoglycaemia, M_1, M_2 and M_3 receptors in the cerebral cortex (36) and M_1 receptor expression in the hippocampus (37) were decreased. In a model of posttraumatic stress, the increase of M_2 expression in the frontal cortex and M_1 expression in the hippocampus show the effect of stress on receptor expression (38). Muscarinic receptor expression is also altered in various pathological conditions including schizophrenia (19), epilepsy (39) and cancer (40). There may such changes occur in the expression of muscarinic receptors leading to the convulsions. Thus, the relationship between receptor expression and fasting and the muscarinic receptor subtype which plays a role in the occurrence of convulsions need to be investigated.

Conclusion

Acetylcholine has prominent functions in the brain. Various physiological and pathological processes involve alterations in muscarinic receptor expression. Studies using M_1 and/or M_2 agonists and antagonists may clarify the underlying mechanisms of convulsions regarding the contribution of cholinergic/anticholinergic activities.

Author contributions: Concept; Literature Search; Writing; Merve Saygı Bacanak, Pharm. PhD.

Conflict of Interest: There is no conflict of interest.

Hakem Değerlendirmesi: Dış Bağımsız.

Yazar Katkıları: Fikir; Literatür taraması; Yazıyı Yazan; Dr. Ecz. Merve Saygı Bacanak

Çıkar Çatışması: Yoktur.

REFERENCES

[1] Levey AI, Kitt CA, Simonds WF, Price DL, Brann MR. Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. J Neurosci 1991; 11: 3218-26.

[2] Lemke TL, Williams DA. Foye's Principles of Medicinal Chemistry. 7th ed. Baltimore and Philadelphia: Lippincott Williams & Wilkins; 2012.

[3] Nathanson NM. Synthesis, trafficking, and localization of muscarinic acetylcholine receptors. Pharmacol Ther 2008; 119: 33-43. Doi: 10.1016/j.pharmthera.2008.04.006

[4] Zhang W, Basile AS, Gomeza J, Volpicelli LA, Levey AI, Wess J. Characterization of central inhibitory muscarinic autoreceptors by the use of muscarinic acetylcholine receptor knock-out mice. J Neurosci 2002; 22: 1709–17.

[5] Vannucchi MG, Pepeu G. Muscarinic receptor modulation of acetylcholine release from rat cerebral cortex and hippocampus. Neurosci Lett 1995; 190: 53-56. Doi: 10.1016/0304-3940(95)11498-L

[6] Suzuki T, Fujimoto K, Oohata H, Kawashima K. Presynaptic M1 muscarinic receptor modulates spontaneous release of acetylcholine from rat basal forebrain slices. Neurosci Lett 1988; 84: 209-12. Doi: 10.1016/0304-3940(88)90409-0

[7] Marchi M, Ruelle A, Andrioli GC, Raiteri M. Pirenzepine-insensitive muscarinic autoreceptors regulate acetylcholine release in human neocortex. Brain Res 1990; 520: 347-50. Doi: 10.1016/0006-8993(90)91728-Y

[8] Rouse ST, Gilmor ML, Levey AI. Differential presynaptic and postsynaptic expression of m1-m4 muscarinic acetylcholine receptors at the perforant pathway/granule cell synapse. Neuroscience 1998; 86: 221-32. Doi: 10.1016/S0306-4522(97)00681-7

[9] Kamsler A, McHugh TJ, Gerber D, Huang SY, Tonegawa S. Presynaptic m1 muscarinic receptors are necessary for mGluR long term depression in the hippocampus. Proc Natl Acad Sci 2010; 107: 1618-23. Doi: 10.1073/pnas.0912540107

[10] Buckley NJ, Bonner TI, Brann MR. Localization of a family of muscarinic receptor mRNAs in rat brain. J Neurosci 1988; 12: 4646-52.

[11] Figueroa KW, Griffin MT, Ehlert FJ. Selectivity of agonists for the active state of M1 to M4 muscarinic receptor subtypes. J Pharmacol Exp Ther 2009; 328: 331-42. Doi: 10.1124/jpet.108.145219

[12] Bymaster FP, Whitesitt CA, Shannon HE, DeLapp N, Ward JS, Calligaro DO, et al. Xanomeline: a selective muscarinic agonist for the treatment of Alzheimer's disease. Drug Dev Res 1997; 40: 158-170.

[13] Wood MD, Murkitt KL, Ho M, Watson JM, Brown F, Hunter AJ, et al. Functional comparison of muscarinic partial agonists at muscarinic receptor subtypes hM1, hM2, hM3, hM4 and hM5 using microphysiometry. Br J Pharmacol 1999; 126: 1620-4.

[14] Witkin JM, Overshiner C, Li X, Catlow JT, Wishart GN, Schober DA, et al. M1 and M2 muscarinic receptor subtypes regulate antidepressant-like

effects of the rapidly acting antidepressant scopolamine. J Pharmacol Exp Ther 2014; 351: 448-56. Doi: 10.1124/jpet.114.216804

[15] Buels KS, Fryer AD. Muscarinic receptor antagonists: effects on pulmonary function. Hofmann FB, editor. Handbook of Experimental Pharmacology. Berlin Heidelberg: Springer-Verlag; 2012.p.317-41. Doi: 10.1007/978-3-642-23274-9_14

[16] Szerb JC, Hadházy P, Dudar JD. Release of [3H]acetylcholine from rat hippocampal slices: effect of septal lesion and of graded concentrations of muscarinic agonists and antagonists. Brain Res 1977; 128: 285-91. Doi: 10.1016/0006-8993(77)90995-7

[17] Langmead CJ, Watson J, Reavill C. Muscarinic acetylcholine receptors as CNS drug targets. Pharmacol Ther 2008; 117: 232–43. Doi: 10.1016/j.pharmthera.2007.09.009

[18] Fisher A. M1 muscarinic agonists target major hallmarks of Alzheimer's disease-the pivotal role of brain M1 receptors. Neurodegener Dis 2008; 5: 237-40. Doi: 10.1159/000113712

[19] Raedler TJ, Bymaster FP, Tandon R, Copolov D, Dean B. Towards a muscarinic hypothesis of schizophrenia. Mol Psychiatry 2007;12: 232-46. Doi: 10.1038/sj.mp.4001924

[20] Greig NH, Reale M, Tata AM. New pharmacological approaches to the cholinergic system: an overview on muscarinic receptor ligands and cholinesterase inhibitors. Recent Pat CNS Drug Discov 2013; 8: 123-41. Doi: 10.2174/1574889811308020003

[21] Eglen RM. Overview of Muscarinic Receptor Subtypes. Hofmann FB, editor. Handbook of Experimental Pharmacology. Berlin Heidelberg: Springer-Verlag; 2012.p.3-28. Doi: 10.1007/978-3-642-23274-9_1

[22] Turski WA, Czuczwar SJ, Kleinrok Z, Turski L. Cholinomimetics produce seizures and brain damage in rats. Experientia 1983; 39: 1408-11. Doi: 10.1007/BF01990130

[23] Cavalheiro EA, Leite JP, Bortolotto ZA, Turski WA, Ikonomidou C, Turski L. Long-term effects of pilocarpine in rats: structural damage of the brain triggers kindling and spontaneous recurrent seizures. Epilepsia 1991; 32: 778–82. Doi: 10.1111/j.1528-1157.1991.tb05533.x

[24] Anderson DR, Harris LW, Bowersox SL, Lennox WJ, Anders JC. Efficacy of injectable anticholinergic drugs against soman-induced convulsive/subconvulsive activity. Drug Chem Toxicol 1994; 17: 139-48. Doi: 10.3109/01480549409014307

[25] Curia G, Longo D, Biagini G, Jones RS, Avoli M. The pilocarpine model of temporal lobe epilepsy. J Neurosci Methods 2008; 172: 143-57. Doi: 10.1016/j.jneumeth.2008.04.019

[26] Hamilton SE, Loose MD, Qi M, Levey AI, Hille B, McKnight GS, et al. Disruption of the M_1 receptor gene ablates muscarinic receptor-dependent M current regulation and seizure activity in mice. Proc Natl Acad Sci 1997; 94: 13311-6. Doi: 10.1073/pnas.94.24.13311

[27] Scorza FA, Arida RM, Naffah-Mazzacoratti Mda G, Scerni DA, Calderazzo L, Cavalheiro EA. The pilocarpine model of epilepsy: what have we learned? An Acad Bras Cienc 2009; 81: 345-65. Doi: 10.1590/S0001-37652009000300003

[28] Enginar N, Nurten A, Yamantürk P, Koyuncuoğlu H. Scopolamine-induced convulsions in food given fasted mice: effects of physostigmine and MK-801. Epilepsy Res 1997; 28: 137-42. Doi: 10.1016/S0920-1211(97)00041-7

[29] Enginar N, Nurten A, Yamantürk-Çelik P, Açıkmeşe B. Scopolamine-induced convulsions in fasted mice after food intake: effects of glucose intake, antimuscarinic activity and anticonvulsant drugs. Neuropharmacology 2005; 49: 293-9. Doi: 10.1016/j.neuropharm.2005.01.032

[30] Enginar N, Nurten A, Ozünal ZG, Zengin A. Scopolamine-induced convulsions in fasted mice after food intake: the effect of duration of food deprivation. Epilepsia 2009; 50: 143-6. Doi: 10.1111/j.1528-1167.2008.01786.x

[31] Enginar N, Yamantürk P, Nurten A, Nurten R, Koyuncuoğlu H. Scopolamine-induced convulsions in fasted mice after food intake: determination of blood glucose levels, [^{3}H]glutamate binding kinetics and antidopaminergic drug effects. Neuropharmacology 2003; 44: 199-205. Doi: 10.1016/S0028-3908(02)00365-9

[32] Enginar N, Nurten A. Seizures triggered by food intake in antimuscarinic-treated fasted animals: evaluation of the experimental findings in terms of similarities to eating-triggered epilepsy. Epilepsia 2010; 51: 80-4. Doi: 10.1111/j.1528-1167.2010.02616.x

[33] Daniels JC, Spehlman R. The convulsant effect of topically applied atropine. Electroencephalogr Clin Neurophysiol 1973; 34: 83-7. Doi: 10.1016/0013-4694(73)90155-7

[34] Tan U, Şenyuva F, Marangoz C. Electrocorticographic effects of topically applied scopolamine. Epilepsia 1978; 19: 223-32. Doi: 10.1111/j.1528-1157.1978.tb04484.x

[35] Weizman A, Bidder M, Fares F, Gavish M. Food deprivation modulates gamma-aminobutyric acid receptors and peripheral benzodiazepine binding sites in rats. Brain Res 1990; 535: 96-100.

[36] Anju TR, Paulose CS. Cortical cholinergic dysregulation as a long-term consequence of neonatal hypoglycemia. Biochem Cell Biol 2015; 93: 47-53. Doi: 10.1139/bcb-2014-0035

[37] Sherin A, Anu J, Peeyush KT, Smijin S, Anitha M, Roshni BT, et al. Cholinergic and GABAergic receptor functional deficit in the hippocampus of insulin-induced hypoglycemic and streptozotocin-induced diabetic rats. Neuroscience 2012; 202: 69-76. Doi: 10.1016/j.neuroscience.2011.11.058

[38] Aykaç A, Aydın B, Cabadak H, Güney Z. The change in muscarinic receptor subtypes in different brain regions of rats treated with fluoxetine or propranolol in a model of post-traumatic stress disorder. Behavioural Brain Res 2012; 232: 124-129. Doi: 10.1016/j.bbr.2012.04.002

[39] Graebenitz S, Kedo O, Speckmann EJ, Gorji A, Panneck H, Hans V, et al. Interictal-like network activity and receptor expression in the epileptic human lateral amygdala. Brain 2011; 134: 2929-47. Doi: 10.1093/brain/awr202

[40] Spindel ER. Muscarinic Receptor Agonists and Antagonists: Effects on Cancer. Hofmann FB, editor. Handbook of Experimental Pharmacology. Berlin Heidelberg: Springer-Verlag; 2012.p.451-68. Doi: 10.1007/978-3-642-23274-9_19

[41] Levey AI. Immunological localization of m1-m5 muscarinic receptor subtypes in peripheral tissues and brain. Life Sci 1993; 52: 441-8.

[42] Nathaniel TI, Umesiri FE, Olajuyigbe F. Role of M1 receptor in the locomotion behavior of the African mole-rat (Cryptomys sp). J Integr Neurosci 2008; 7: 1-16.

[43] Ma L, Seager MA, Wittmann M, Jacobson M, Bickel D, Burno M, et al. Selective activation of the M1 muscarinic acetylcholine receptor achieved by allosteric potentiation. Proc Natl Acad Sci 2009; 106: 15950–5. Doi: 10.1073/pnas.0900903106

[44] Coleman CG, Lydic R, Baghdoyan HA. M2 muscarinic receptors in pontine reticular formation of C57BL/6J mouse contribute to rapid eye movement sleep generation. Neuroscience 2004; 126: 821–30. Doi: 10.1016/j.neuroscience.2004.04.029

[45] Wess J, Duttaroy A, Gomeza J, Zhang W, Yamada M, Felder CC, et al. Muscarinic receptor subtypes mediating central and peripheral antinociception studied with muscarinic receptor knockout mice: a review. Life Sci 2003; 72: 2047-54. Doi: 10.1016/S0024-3205(03)00082-1

[46] Gautam D, Jeon J, Starost MF, Han SJ, Hamdan FF, Cui Y, et al. Neuronal M3 muscarinic acetylcholine receptors are essential for

somatotroph proliferation and normal somatic growth. Proc Natl Acad Sci 2009; 106: 6398-403. Doi: 10.1073/pnas.0900977106

[47] Poulin B, Butcher A, McWilliams P, Bourgognon JM, Pawlak R, Kong KC, et al. The M3-muscarinic receptor regulates learning and memory in a receptor phosphorylation/arrestin-dependent manner. Proc Natl Acad Sci 2010; 107: 9440-5. Doi: 10.1073/pnas.0914801107

[48] Yamada M, Lamping KG, Duttaroy A, Zhang W, Cui Y, Bymaster FP, et al. Cholinergic dilation of cerebral blood vessels is abolished in M(5) muscarinic acetylcholine receptor knockout mice. Proc Natl Acad Sci 2001; 98: 14096-101. Doi: 10.1073/pnas.251542998

[49] Foster DJ, Gentry PR, Lizardi-Ortiz JE, Bridges TM, Wood MR, Niswender CM, et al. M5 receptor activation produces opposing physiological outcomes in dopamine neurons depending on the receptor's location. J Neurosci 2014; 34: 3253-62. Doi: 10.1523/JNEUROSCI.4896-13.2014

[50] Jones CK, Byun N, Bubser M. Muscarinic and nicotinic acetylcholine receptor agonists and allosteric modulators for the treatment of schizophrenia. Neuropsychopharmacology 2012; 37: 16-42. Doi: 10.1038/npp.2011.199