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Rapid eye movement (REM) sleep deprivation reduces rat frontal cortex acetylcholinesterase (EC 3.1.1.7) activity

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Abstract

Rapid eye movement (REM) sleep deprivation induces several behavioral changes. Among these, a decrease in yawning behavior produced by low doses of cholinergic agonists is observed which indicates a change in brain cholinergic neurotransmission after REM sleep deprivation. Acetylcholinesterase (Achase) controls acetylcholine (Ach) availability in the synaptic cleft. Therefore, altered Achase activity may lead to a change in Ach availability at the receptor level which, in turn, may result in modification of cholinergic neurotransmission. To determine if REM sleep deprivation would change the activity of Achase, male Wistar rats, 3 months old, weighing 250-300 g, were deprived of REM sleep for 96 h by the flower-pot technique (N = 12). Two additional groups, a home-cage control (N = 6) and a large platform control (N = 6), were also used. Achase was measured in the frontal cortex using two different methods to obtain the enzyme activity. One method consisted of the obtention of total (900 *g* supernatant), membrane-bound (100,000 *g* pellet) and soluble (100,000 *g* supernatant) Achase, and the other method consisted of the obtention of a fraction (40,000 *g* pellet) enriched in synaptic membrane-bound enzyme. In both preparations, REM sleep deprivation induced a significant decrease in rat frontal cortex Achase activity when compared to both home-cage and large platform controls. REM sleep deprivation induced a significant decrease of 16% in the membrane-bound Achase activity (nmol thiocholine formed min^{-1} mg $protein^{-1}$) in the 100,000 *g* pellet enzyme preparation (home-cage group 152.1 ± 5.7, large platform group 152.7 ± 24.9 and REM sleep-deprived group 127.9 ± 13.8). There was no difference in the soluble enzyme activity. REM sleep deprivation also induced a significant decrease of 20% in the enriched synaptic membrane-bound Achase activity (home-cage group 126.4 ± 21.5, large platform group 127.8 ± 20.4, REM sleep-deprived group 102.8 ± 14.2). Our results suggest that REM sleep deprivation changes Ach availability at the level of its receptors through a decrease in Achase activity.

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- REM sleep deprivation
- Frontal cortex
- Acetylcholinesterase

Introduction

Rapid eye movement (REM) sleep deprivation induces several behavioral changes in rats (1), among them a decrease in yawning behavior induced by low doses of cholinergic agonists (2,3) indicating alteration in cholinergic sensitivity. In humans, it is claimed that REM sleep deprivation has an antidepressant effect (4) and cholinergic neurotransmission has been proposed to be implicated in depression (5,6). These findings suggest a change of cholinergic neurotransmission after REM sleep deprivation.

The frontal cortex is involved in many aspects of behavior. Lesions in this region result in changes of social and affective behaviors (7). Among the afferents to the frontal cortex the cholinergic input from the basal forebrain neurons, especially those of the nucleus basalis magnocellularis (NBM), has been receiving great attention (8,9). A considerable amount of data show that lesions of the NBM result in decreased cholinergic markers in the frontal cortex, such as acetylcholine (Ach), choline uptake, choline acetyltransferase (CAT) and acetylcholinesterase (Achase) activity (10-17). Moreover, since some authors report a decrease in REM sleep in animals with NBM lesions (18,19), it is of interest to evaluate the cholinergic projections from NBM after REM sleep deprivation.

Acetylcholinesterase (EC 3.l.l.7) is an important constituent of cholinergic neurotransmission (20). Achase hydrolyzes Ach, terminating its action at the synaptic cleft. This enzyme is used as a marker for monitoring NBM lesions and the available data have shown a decrease in enzyme activity in the frontal cortex of lesioned rats (10-17). On the other hand, inhibition of Achase leads to down-regulation of cholinergic receptors (15), indicating that changes in Achase may result in changes in Ach availability at the level of its receptors.

We measured Achase activity in rat frontal cortex after REM sleep deprivation in order to detect possible changes in cholinergic neurotransmission. Our results showed a decrease in Achase activity, indicating that REM sleep deprivation could change cholinergic neurotransmission.

Material and Methods

Subjects and REM sleep deprivation

Male Wistar rats bred in our colony, 3 months old and weighing 250-300 g, were used in this study. After weaning, rats were kept in wire-mesh cages in groups of 3 in a room with a controlled light-dark cycle (lights on from 7:00 a.m. to 7:00 p.m.) and temperature (22 ± 2°C). Animals had free access to tap water and Purina® lab chow until sacrifice. Handling of animals consisted of room and cage cleaning. REM sleep deprivation started at 9:00 a.m. Animals were placed individually on a small flower-pot in water tanks for 96 h (2,3,21,22). The flower-pot was 5.5 cm in diameter and surrounded by water up to 2 cm below its surface. Control rats were kept individually in their home cages for 96 h. Another control group was submitted to the same conditions as the REM sleep-deprived group, except that this control group was placed on large platforms, measuring 14 cm in diameter, surrounded by water up to 2 cm below the top.

During the deprivation period all animals had free access to water and food. The deprivation length was chosen based on behavioral and biochemical effects produced by 96 h of REM sleep deprivation, already described in the literature (2,3,21,22).

Tissue preparation

Total and frontal cortices were obtained from different animals. After decapitation brains were excised and kept on a cooled Petri dish on crushed ice. Brains were washed superficially with isotonic saline to remove

blood. Cortices were immediately dissected and the tissue weighed and kept in cold sucrose 0.32 M, pH 7.4, or 0.25 M, pH 7.4, depending on the homogenate to be prepared.

Homogenate preparation

Homogenates (5% w/v) were prepared using glass homogenizer tubes and a motor-driven Teflon™ pestle. Two homogenates were prepared: one in 0.32 M sucrose, according to Chubb and Smith (23), with centrifugation of the material at 900 *g* for 10 min at 0°C. The supernatant was collected and centrifuged at 100,000 *g* for 60 min at 4°C. The supernatant from this step was the source of soluble Achase and the resulting pellet was resuspended in the original volume. The resuspended pellet preparation was the source of membrane-bound Achase. Enzyme activity was also measured in the 900 *g* supernatant. Homogenates were kept at -20°C until the time for assay.

A second homogenate was prepared in 0.25 M sucrose (5% w/v) according to Swann (24). The homogenate was first centrifuged at 900 *g* for 10 min at 0°C. The supernatant was centrifuged at 40,000*g* for 60 min at 4°C and the pellet was resuspended in 0.25 M sucrose and centrifuged under the same conditions; this procedure was repeated one more time. Finally, the sediment was resuspended and kept at -20°C until the time for assay.

The homogenates obtained by the first method (23) contained all membranes in the 100,000 *g* fraction except those discarded in the 900 *g* centrifugation, and differed from the preparation obtained in the second method (24), which was rich in nerve endings.

Determination of Achase activity

Achase activity was determined by the method of Ellman et al. (25) adapted for microassay. Acetylthiocholine was used as substrate at a final concentration of 1 mM. All materials, including reagents and homogenates, were kept on crushed ice before incubation. Enzyme activity was determined in duplicate for both samples and blanks. One hundred µl of buffer-substrate (0.1 M sodium phosphate buffer, pH 8.0, plus acetylthiocholine iodide) was pipetted into a microtube and 5 µl of homogenate was added. Blanks were obtained by adding 15 µl of 2.4 N perchloric acid to the tubes before incubation. The tubes were incubated for 30 min in a shaking water bath at 37°C. After the addition of perchloric acid, the enzyme activity tubes were centrifuged at 2,250 *g* for 15 min at 0°C. An aliquot of 50 µl was pipetted into another tube and 500 µl of Ellman's reagent was added. After 15 min, samples were read in a spectrophotometer using microcuvettes at 412 nm. Proteins were assayed by the method of Lowry et al. (26) using bovine serum albumin as standard. The activity of Achase is reported as nmol thiocholine formed min^{-1} mg $protein^{-1}$.

The assay was adjusted to allow the reaction to occur in the linear region for both tissue concentration and incubation time. To test the contribution of butyrylcholinesterase to Ach hydrolysis the enzyme activity was measured using butyrylthiocholine as the substrate at 1 mM final concentration. Since the activity was very low (<5%) as also shown by other authors (27), no butyrylcholinesterase inhibitor was used.

Reagents

All reagents used were of analytical grade either from Sigma Chemical Co. (St. Louis, MO) or Merck Co. (São Paulo, Brazil). Twice distilled water prepared in an all-glass-apparatus was used for the assays.

Statistical analysis

One-way analysis of variance (ANOVA) followed by *post hoc* Duncan's multiple range test was used to detect differences among

and between groups, respectively. The level of significance was set at P≤0.05, two-tailed.

Results

Table 1 and Figure 1 show the results obtained for Achase activity after REM sleep deprivation using different extraction procedures. As can be seen, the enzyme activity was decreased after REM sleep deprivation in both homogenates. One-way ANOVA of the data in Table 1 showed a significant difference among groups in the 900 *g* homogenate ($F_{2,21}$ = 4.61, P = 0.04) and the 100,000 *g* pellet ($F_{2,21}$ = 7.4, P = 0.008), but no difference in the 100,000 *g* supernatant ($F_{2,20}$ = 3.80, P = 0.06). *Post hoc* analysis showed that in the 900 *g* homogenate the Achase activity of REM sleep-deprived rats was significantly lower than in the home-cage group (P = 0.04). In the 100,000*g* pellet fraction, Achase activity after REM sleep deprivation was also significantly lower than in the home-cage (P = 0.006) and the large platform controls (P = 0.02).

One-way ANOVA of the data in Figure 1 also showed a significant difference ($F_{2,21}$ = 5.59, P = 0.01). Comparison between groups showed that the Achase activity was significantly lower in REM sleep-deprived rats than in the home-cage group (P = 0.01) and the large platform group (P = 0.05).

Using the same experimental procedure as for the frontal cortex, Achase activity was also examined in total cerebral cortex. No significant changes were observed in Achase activity in this brain region after REM sleep deprivation (data not shown).

Table 1 - Effect of REM sleep deprivation (REMSD) on the activity of Achase from fractions of rat frontal cortex.

Achase activity is reported as nmol thiocholine formed min^{-1} mg $protein^{-1}$. Final acetylthiocholine concentration in the assay was 1 mM. Proteins in the 900 *g* supernatant ranged from 11-15 µg in 5 µl of homogenate, in the pellet from 8.5-10 µg, and in the 100,000 *g* supernatant from 3.1-3.6 µg. Values are reported as mean ± SD. [a]P = 0.04, REMSD x home-cage; [b]P = 0.006, REMSD x home-cage; [c]P = 0.02, REMSD x large platform (Duncan's multiple range test).

Group	Fraction		
	Supernatant (900 *g*)	Pellet (100,000 *g*)	Supernatant (100,000 *g*)
Home-cage	176.3 ± 26 N = 6	152.1 ± 5.7 N = 6	153.1 ± 7.9 N = 6
Large platform	184.9 ± 39 N = 6	152.7 ± 24.9 N = 6	143.3 ± 28.1 N = 6
REMSD	148.2 ± 39[a] N = 12	127.9 ± 13.8[b,c] N = 12	126.4 ± 19.0 N = 12

Discussion

The results obtained in this study showed that REM sleep deprivation resulted in a consistent decrease in membrane-bound Achase activity in rat frontal cortex, but not in total cerebral cortex (data not shown). This change was also detected when REM sleep-deprived animals were compared with the large platform group, suggesting a specific effect of REM sleep deprivation rather than a general effect of stress on Achase activity in the rat frontal cortex.

REM sleep deprivation induced similar effects on membrane-bound Achase activity in both enzyme sources assayed. The preparation shown in Table 1 allowed to assay total, all membrane-bound and soluble enzyme activity and the results depicted in Figure 1 are from a fraction enriched in nerve endings. REM sleep deprivation induced a significant decrease in membrane-bound (16%) and no change in soluble enzyme (Table 1). It also induced a decrease (20%) in Achase activity in the synaptic enriched preparation (Figure 1) which contains only membrane-bound enzyme. Achase has multiple molecular forms that differ in several aspects including subcellular localization (20). The results obtained for the two enzyme sources indicate that the decrease in Achase observed after REM sleep deprivation may be located on membranes from nerve endings and probably involves a decrease in the globular G_4 form which is

mostly bound to synaptic membranes (20).

Several data show that lesions of neurons of the NBM induce a decrease in Achase activity in the frontal cortex (10-17). This indicates that NBM is a major cholinergic afferent to the frontal cortex. However, it should be mentioned that cholinergic innervation in the rat frontal cortex does not originate exclusively from the NBM, but also from neurons within the cerebral cortex (13).

Achase controls Ach levels in the synaptic cleft by its hydrolytic activity on Ach molecules (20) and a decrease in its activity may account for a higher Ach availability at the level of its receptors. This, in turn, can lead to receptor down-regulation, as shown after administration of Achase inhibitors (15). Following this rationale, Ach receptor down-regulation might be expected after REM sleep deprivation. In fact, in an autoradiography study Nunes Jr. et al. (28) recently showed decreased M_2 cholinergic receptors in several brain regions, but did not detect any change in this particular receptor subtype in the frontal cortex of REM sleep-deprived rats. Other investigators have also failed to find any change in frontal cortex M_1/M_2 or nicotinic receptors after 10 days of total sleep deprivation (29). However, due to the well-known diversity of cholinergic receptors, including diversity within the M_2 class of receptor, and conflicting reports about receptor type in the frontal cortex (30-32), one should not reject the possibility of modification in either muscarinic or nicotinic cholinergic receptors in this brain region after REM sleep deprivation.

During REM sleep, Ach is increased on the surface of cerebral cortex of cats (33). In contrast, after REM sleep deprivation Ach levels are decreased in the rat telencephalon (34,35). These data, taken together with ours, indicate a decrease in cholinergic activity after REM sleep deprivation, a situation possibly resembling the effect of NBM lesions on cholinergic neurotransmission. The decrease in REM sleep in animals with NBM lesions (18,19) also favors this possibility.

The decrease in cholinergic neurotransmission induced by REM sleep deprivation would be expected to induce an up-regulation of post-synaptic cholinergic receptors in the frontal cortex. Earlier data on NBM lesions which also induce a decrease in Achase activity showed either a reduction in muscarinic receptor number (11) or no change (10,15). More recent data from NBM-lesioned rats are inconclusive regarding changes in frontal cortex cholinergic receptors. The cholinergic muscarinic receptor M_1 subtype was shown to be decreased (36) or not changed (37,38), while muscarinic receptor M_2 was shown to be increased (37) or decreased (38). Therefore, these data show that a decrease in cholinergic neurotransmission, and its association with changes in cholinergic receptors, is not a simple phenomenon. These data also explain the conflicting reports on cholinergic receptors after REM sleep deprivation.

The nucleus basalis magnocellularis accounts for 70-80% of the cholinergic innervation to the cortex (39). Moreover, different cortical areas receive their major cholinergic input from individual sectors of the

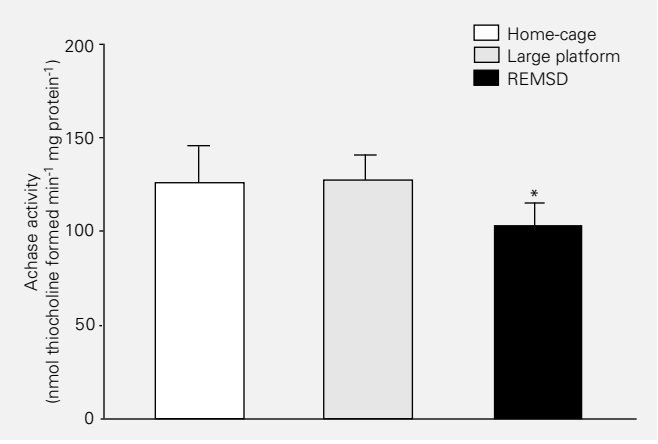

Figure 1 - Activity of bound Achase from enriched synaptic membrane preparation of rat frontal cortex after REM sleep deprivation (REMSD). Achase activity is reported as nmol thiocholine formed min^{-1} mg $protein^{-1}$. Data are reported as mean ± SD. *P = 0.01 for REM sleep-deprived (N = 12) *vs* home-cage group (N = 6) and *P = 0.05 *vs* large platform group (N = 6).

NBM (40). Additional input to the cortex is received from neurons in the midbrain reticular system and the dorsolateral pontine tegmentum. The remainder of cholinergic innervation is most likely derived from intrinsic neurons. NBM lesions (a procedure that is more severe than REM sleep deprivation) induce a large decrease in cortical Achase activity. Data in the literature have shown that this decrease is specific for some cortical areas, with Achase activity decreasing in the frontal and parietal but not in the occipital or temporal cortex (16). The decrease in Achase activity in the frontal cortex after REM sleep deprivation ranged from 16 to 20%. On the other hand, the decrease in Achase activity after NBM lesion reaches up to 50%. Achase does not have a uniform distribution in the brain, including cortical areas (41). This clearly indicates that the overall Achase activity in whole cerebral cortex receives different contributions from each particular cortical region. It is possible that the decrease in Achase activity induced by REM sleep deprivation may be specific for the frontal cortical area. Hence, the small but significant decrease after REM sleep deprivation may have not been detectable when the whole cerebral cortex was assayed.

The consequences of decreased Achase activity in the frontal cortex induced by REM sleep deprivation for cholinergic neurotransmission could be interpreted in two ways, as done above. Although the available data on cholinergic receptors after REM sleep deprivation or NBM lesion do not allow, at present, a final conclusion regarding the relationship between the alteration in Achase activity induced by REM sleep deprivation and Ach levels in the synaptic cleft of the frontal cortex, some other experimental evidence may indicate that the second interpretation is more likely. A 50% decrease in the Achase activity of rat hindlimb muscle by blocking the conduction of action potentials along the sciatic nerve with tetrodotoxin was reported (42). On the other hand, Ach seems to increase the Achase synthesis in cultures of chicken embryo muscle (43). Taken together with the observed decrease in Ach levels in the telencephalon after REM sleep deprivation (34,35), these data suggest that the decrease in Achase activity is the result of a decrease in cholinergic neurotransmission in the frontal cortex after REM sleep deprivation.

References

1. Carlini EA (1983). REM sleep deprivation and dopamine in the CNS. *Reviews in Pure and Applied Pharmacological Sciences*, 4: 1-25.
2. Neumann BG, Troncone LRP, Braz S & Tufik S (1990). Modifications on dopaminergic and cholinergic systems induced by the water tank technique: Analysis through yawning behavior. *Archives Internationales de Pharmacodynamie et de Therapie*, 308: 32-38.
3. Tufik S, Troncone LRP, Braz S, Silva-Filho AR & Neumann B (1987). Does REM sleep deprivation induce subsensitivity of presynaptic dopamine or postsynaptic acetylcholine receptors in the rat brain? *European Journal of Pharmacology*, 140: 215-219.
4. Vogel GW, Buffenstein A, Minter K & Hennessey A (1990). Drug effects on REM sleep and on endogenous depression. *Neuroscience and Biobehavioral Reviews*, 14: 49-69.
5. Dilsaver SC (1986). Cholinergic mechanisms in depression. *Brain Research Reviews*, 11: 285-316.
6. Janowsky DS, El-Yousef MK, Davis JM & Sekerke JH (1972). A cholinergic-adrenergic hypothesis of mania and depression. *Lancet*, 2: 632-635.
7. Kolb B (1984). Functions of the frontal cortex of the rat: A comparative review. *Brain Research Reviews*, 8: 65-98.
8. Luiten PGM, Gaykema RPA, Traber J & Spencer Jr DG (1987). Cortical projection patterns of magnocellular basal nucleus subdivisions as revealed by anterogradely transported *Phaseolus vulgaris* leucoagglutinin. *Brain Research*, 413: 229-250.
9. Woolf NJ (1991). Cholinergic systems in mammalian brain and spinal cord. *Progress in Neurobiology*, 37: 475-524.
10. Altman HJ, Crosland RD, Jenden DJ & Berman RF (1985). Further characterizations of the nature of the behavioral and neurochemical effects of lesions to the nucleus basalis of Meynert in the rat. *Neurobiology of Aging*, 6: 125-130.

11. Belleroche J de, Gardiner IM, Hamilton MH & Birdsall NJM (1985). Analysis of muscarinic receptor concentration and subtypes following lesion of rat substantia innominata. *Brain Research*, 340: 201-209.
12. Gardiner IM, de Belleroche J, Premi BK & Hamilton MH (1987). Effect of lesion of the nucleus basalis of rat on acetylcholine release in cerebral cortex: Time course of compensatory events. *Brain Research*, 407: 263-271.
13. Johnston MV, McKinney M & Coyle JT (1981). Neocortical cholinergic innervation: A description of extrinsic and intrinsic components in the rat. *Experimental Brain Research*, 43: 159-172.
14. Lehmann J, Nagy JI, Atmadyn S & Fibiger HC (1980). The nucleus basalis magnocellularis: The origin of a cholinergic projection to the neocortex of the rat. *Neuroscience*, 5: 1161-1174.
15. Mandel RJ, Chen AD, Connor DJ & Thal LJ (1989). Continuous physostigmine infusion in rats with excitotoxic lesions of the nucleus basalis magnocellularis: Effects on performance in the water maze task and cortical cholinergic markers. *Journal of Pharmacology and Experimental Therapeutics*, 251: 612-619.
16. Miyamoto M, Kato J, Narumi S & Nagaoka A (1987). Characteristics of memory impairment following lesioning of the basal forebrain and medial septal nucleus in rats. *Brain Research*, 419: 19-31.
17. Riekkinen Jr P, Miettinen R, Rummukainen J, Pitkanen A, Paljarvi L & Riekkinen P (1990). The effects of lesioning the basal forebrain cholinergic neurones on CSF AChE activity. *Neuroscience Research Communications*, 6: 37-43.
18. Stone WS & Gold PE (1988). Sleep and memory relationships in intact old and amnestic young rats. *Neurobiology of Aging*, 9: 719-727.
19. Stone WS, Altman HJ, Berman RF, Caldwell DF & Kilbey MM (1989). Association of sleep parameters and memory in intact old rats and young rats with lesions in the nucleus basalis magnocellularis. *Behavioral Neuroscience*, 103: 755-764.
20. Brimijoin S (1983). Molecular forms of acetylcholinesterase in brain, nerve and muscle: Nature, localization and dynamics. *Progress in Neurobiology*, 21: 291-322.
21. Troncone LRP, Braz S, Benedito MAC & Tufik S (1986). REM sleep deprivation induces a decrease in norepinephrine-stimulated ^{3}H-cyclic AMP accumulation in slices from rat brain. *Pharmacology, Biochemistry and Behavior*, 25: 223-225.
22. Zwicker AP & Calil HM (1986). The effects of REM sleep deprivation on striatal dopamine receptor sites. *Pharmacology, Biochemistry and Behavior*, 24: 809-812.
23. Chubb IW & Smith AD (1975). Isoenzymes of soluble and membrane-bound acetylcholinesterase in bovine splanchnic nerve and adrenal medulla. *Proceedings of the Royal Society*, *Series B*, 191: 245-261.
24. Swann AC (1988). Norepinephrine and (Na^+,K^+)-ATPase: evidence for stabilization by lithium or imipramine. *Neuropharmacology*, 27: 261-267.
25. Ellman GL, Courtney KD, Andres-Jr V & Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7: 88-95.
26. Lowry OH, Rosebrough NJ, Farr AL & Randall RJ (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193: 265-275.
27. Hobbiger F & Lancaster R (1971). The determination of acetylcholinesterase activity of brain slices and its significance in studies of extracellular acetylcholinesterase. *Journal of Neurochemistry*, 18: 1741-1749.
28. Nunes Jr GP, Tufik S & Nobrega JN (1994). Decreased muscarinic receptor binding in rat brain after paradoxical sleep deprivation: an autoradiographic study. *Brain Research*, 645: 247-252.
29. Tsai L, Bergmann BM, Perry BD & Rechtschaffen A (1994). Effects of chronic sleep deprivation on central cholinergic receptors in rat brain. *Brain Research*, 642: 95-103.
30. Araújo DM, Lapchak PA, Regenold W & Quirion R (1989). Characterization of [^{3}H] AF-DX 116 binding sites in the rat brain: Evidence for heterogeneity of muscarinic M_2 receptor sites. *Synapse*, 4: 106-114.
31. Mash DC & Potter LT (1986). Autoradiographic localization of M_1 and M_2 muscarine receptors in the rat brain. *Neuroscience*, 19: 551-564.
32. Spencer Jr DG, Horvath E & Traber J (1986). Direct autoradiographic determination of M_1 and M_2 muscarinic acetylcholine receptor distribution in the rat brain: Relation to cholinergic nuclei and projections. *Brain Research*, 380: 59-68.
33. Jasper HH & Tesser J (1970). Acetylcholine liberation from cerebral cortex during paradoxical (REM) sleep. *Science*, 172: 601-602.
34. Bowers Jr MB, Hartman EL & Freedman DX (1966). Sleep deprivation and brain acetylcholine. *Science*, 153: 1416-1417.
35. Tsuchiya K, Toru M & Kobayashi T (1969). Sleep deprivation: changes of monoamines and acetylcholine in rat brain. *Life Sciences*, 8: 867-873.
36. Dawson VL, Hunt ME & Wamsley JK (1992). Alteration in cortical muscarinic receptors following cholinotoxin (AF64A) lesion of the rat nucleus basalis magnocellularis. *Neurobiology of Aging*, 13: 25-32.
37. Bogdanovic N, Islam A, Nilsson L, Bergstrom L, Winblad B & Adem A (1993). Effects of nucleus basalis lesion on muscarinic receptor subtypes. *Experimental Brain Research*, 97: 225-232.
38. Schlieb R, Ferst T, Rossner S & Bigl V (1994). Receptor function in cortical rat brain regions after lesion of nucleus basalis. *Journal of Neural Transmission*, 44: 195-208.
39. Dekker JAM, Connor DJ & Thal LJ (1991). The role of cholinergic projections from the nucleus basalis in memory. *Neuroscience and Biobehavioral Reviews*, 15: 299-317.
40. Mesulam MM (1995). Structure and function of cholinergic pathways in the cerebral cortex, limbic system, basal ganglia, and thalamus of the human brain. In: Bloom FE & Kupfer DJ (Editors), *Psychopharmacology*: *The Fourth Generation of Progress*. Raven Press, New York, 135-146.
41. Hoover DB, Muth EA & Jacobowitz DM (1978). A mapping of the distribution of acetylcholine, choline acetyltransferase and acetylcholinesterase in discrete areas of rat brain. *Brain Research*, 153: 295-306.
42. Butler IJ, Drachman DB & Goldberg AM (1978). The effect of disuse on cholinergic enzymes. *Journal of Physiology*, 274: 593-600.
43. Massoulié J & Bon S (1982). The molecular forms of cholinesterase and acetylcholinesterase in vertebrates. *Annual Review of Neuroscience*, 5: 57-106.