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Brain Stem and Cerebral Cortex Histamine Concentrations in the Rem Sleep Deprived Rats

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Abstract: Histamine is a biogenic amine widely distributed in the central nervous system that acts in a neuroregulatory role in controlling the waking state. The aim of this study was to determine whether 120 h of REM sleep deprivation (REMSD) causes changes in histamine concentrations in rat brain stems and cerebral cortex areas that are involved in the homeostatic regulation of sleep.

A number of rats were subjected to 120 h of REM sleep deprivation using the flower pot water tank technique. This method used to induce REMSD is believed to be stressful. To control for the stress caused by the water environment, a tank control group (K II) was included, in which the animals could reside comfortably on a large platform in the water tank. The rats were divided into three groups: 1- The REMSD group resided in a water tank for 120 h (RD), 2- The tank control group resided in the water tank on a large platform for 120 h (K II), 3- The cage controls remained in their home cages for the entire duration of the study (K I). The brains were examined for histamine in the brain stem and cerebral cortex by the fluorometric method. The data was analysed through the Mann-Whitney U test.

Histamine concentrations were significantly increased in the brain stem of the RD and K II groups compared to K I group rats. These results suggest that an increase in brain stem histamine levels was associated with increased histaminergic neuron activity in rats exposed to the water tank treatment for 120 h.

Key Words: REM sleep, deprivation, histamine, brain stem, cerebral cortex

Introduction

REM sleep deprivation (REMSD) is a potent stressor for rats, and can even be fatal, since they survive only 16-54 days of REMSD (1, 2). Studies show that REMSD causes behavioural abnormalities, disturbances of thermoregulation, as well as hormonal and metabolic changes in rats (1-4). These results are probably related to the changes in neurotransmitter activity in the brain. REMSD increases monoaminergic (noradrenergic and serotonergic) turnover and their metabolites in the entire rat brain (5). Recently, a significant increase in the concentration of dopamine metabolites was observed in the striatum, while the concentration of serotonin and its metabolites was reduced in the frontal and parietal cortex in 96 h REMSD rats (6). These neurotransmitters are involved in mediating higher brain functions such as the attention mechanism, sensory information processing and locomotor activity, which are severely affected in REMD (6).

Histamine acts as a neurotransmitter and neuromodulator regulating neuroendocrine and neuroimmune functions, circadian rhythms, the sleep wakefulness cycle, body temperature, centrally-mediated neurovegatative functions, cerebrovascular control, general behaviour and learning (7-9). The histaminergic neurons are located exclusively in the tuberomammillary nucleus in the posterior basal hypothalamus, and send their varicose fibres to almost all regions of the brain (8, 9). The known neuroanatomical connections of the histaminergic pathway resemble those of the ascending noradrenergic and serotonergic components of the reticular activating system of the brain stem (10). The posterior hypothalamus also sends descending projections to the mesopontine tegmentum, which contain cholinergic, noradrenergic, and other neuronal populations playing a key role in the generation of wakefulness and paradoxical sleep (11). It was also demonstrated that histaminergic neurons send fibre projections to the basal forebrain and substantia innominata, which contain magnocellular cholinergic and noncholinergic neurons projecting to the neocortex that probably play an important role in cortical activation (11).

These observations suggest that histaminergic neurons may promote cortical activation and wakefulness (11).

Both neurochemical and electrophysiologic studies indicate that histaminergic neuronal activity is maximal during periods of wakefulness. An increased release of histamine in the posterior hypothalamus of monkeys occurs on waking and is maintained during the waking episode (11). Microinjection of histamine in the preoptic area increased the wakefulness and decreased the slow wave and REM sleep (12). Systemically administered H1 receptor antagonists are sedative and increase sleep in cats and dogs. Histamine synthesis inhibition leads to a significant increase in REM sleep (10).

The primary objective of this experiment was to determine how histamine levels are modified in the brain tissues of rats during a 120 h REMSD period. It was expected that histaminergic activity would be increased during prolonged waking.

Materials and Methods

A total of 18 male Wistar-Albino rats, weighting 220-240g, were used. They were acclimatised during the 3 days prior to the beginning of the study and housed individually in cages with ad libitum access to food and water. Rats were subjected to REMSD for 120 h by the water tank method (13). In the flower pot technique, rats are placed on a small platform over the water and the loss of muscle tone during REM sleep causes them to fall into the water. This technique may result in some loss of non-REM sleep in addition to REM sleep deprivation (14). The flower pot technique also involves some non-specific stress that is dependent upon the ratio of animal weight to the surface area of the platform (14).

The rats were divided into three groups:

1- The REMSD group (RD) (n:6), that resided on a 6.5 cm diameter platform in the water tank for 120 h. The platform was 5 cm above the water. To control for the effects of the stress associated with the water tank, a tank control group was included.

2- The tank control group (K II) (n:6) consisted of rats that resided on an 11 cm diameter platform during the sham deprivation

3- The cage control (K I) (n:6) consisted of rats that were kept in their cages

The chamber was provided with food tubes and water supplies and a 12/12 h light/dark rhythm was maintained. After a 120 h deprivation period, the animals were killed by exsanguination under ketamine hydrocholoride anaesthesia in the morning and were decapitated immediately. The brain stem and cerebral cortex were rapidly dissected. Tissue samples were homogenised with perchloric acid, the homogenate was centrifuged and the supernatant was analysed. Histamine was determined by the fluorometric method (15). The eluate was derived with o-phthalaldehyde. Fluorescence intensity was at 450 nm with excitation with 360 nm in a spectrofluorometer (15).

Statistical analysis: Data analysis was carried out by using SPSS 9.01. Histamine concentrations for experimental and control groups were analysed by using the non-parametric, Mann-Whitney U test. P values of less than 0.05 were considered to be statistically significant.

Results

Histamine levels of the brain stem and cerebral cortex are shown in the Table. Brain-stem histamine concentration of REMS (RD) and sham deprived (K II) rats were significantly higher than that of the cage controls (K I) ($p< 0.005$, 0.05). In the K II group, when compared to the RD group, a statistically significant difference was also found between the groups ($p< 0.005$). There was no significant difference between histamine concentrations in the cerebral cortex of the different groups.

Discussion

In the present study, histamine levels were found to be higher in RD and K II groups than in the K I group. However, cerebral cortex histamine concentrations did not increase significantly in either tank group. It is possible that the increase in histamine concentrations in the brain stems of RD and K II groups is more closely associated with an increase in wakefulness or adaptation to the water tank condition or increased alertness to the environmental stimuli in those rats. Wakefulness could be related to the enhanced release of histamine.

Rats subjected to either total or REMSD chronically have demonstrated an increase in food intake, but also a weight loss due to increased energy expenditure (16-18).

Table: Concentration of histamine in the brain stem and the cerebral cortex of RD, K I and K II groups (mg/g tissue wet weight).

Groups	Histamine Concentration (mg/g tissue)	
	Brain stem Mean±SEM	Cerebral Cortex Mean±SEM
RD (n:6)	9.70±0.74[a]	4.30±0.38
K II (n:6)	4.04±0.67[b]	4.22±0.81
K I (n:6)	2.27±0.34[c]	4.31±0.29

RD: REM sleep deprivation, K II: tank control, K I: cage control
(a, b), (a, c) $p<0.005$ (b, c) $p< 0.05$

Most changes in neuroendocrine parameters appear to be responses to metabolic demands (19, 20).

In rats, sleep deprivation results in a variety of behavioural changes. REMSD rats show increased aggression, and locomotor and exploratory activity, but decreased emotionality, less fear and greater sensitivity to environmental stimuli than controls, the mechanism for which is largely unknown. In addition to its physiological effects, sleep deprivation impairs cognitive function (6, 14).

The known neurochemical connections of the histaminergic pathway resemble those of ascending noradrenergic and serotonergic components of the reticular activator system (10). Histaminergic neurons also have widespread projection to the thalamus and neocortex, and are tonically active during wakefulness (11).

Histamine plays an essential role in the maintenance of wakefulness. Histamine synthesis inhibition leads to a significant increase in REM sleep. During deep slow–wave sleep and REM sleep most of the hypothalamic histaminergic neurons become silent. Histamine levels reach a minimum during the dark phase (10).

After lesions of the ascending histaminergic pathway at the hypothalamic level, the histamine content in cerebral cortex transiently rises, which may reflect an impaired release resulting from the interruption of impulse flow (21). Porkka-Heiskanen et al. (1994) found that the histamine concentrations in the hypothalamus did not differ between a REMSD group and other control groups after a 72 h REMSD period. In the anterior hypothalamus, however, histamine levels increased during rebound sleep only in the REMSD rats (13).

Other alternative explanations for increased histamine concentration in the brain stem of both RD and K II groups were consistent with an activation of histaminergic neuron activity or a decrease of the histamine metabolism in the rats exposed to the water tank treatment for 120 h. Even though REMS is effectively deprived by the flower pot technique, changes induced by this procedure are not necessarily a consequence of selective REMSD. The influence of stress by the restriction of motor activity and repeated exposure to the water tank cannot be excluded.

On the other hand, histamine may be important in the response of animals to stress. Various stressful conditions affect histamine turnover in the brain. The systemic administration of histamine elicits an increase in plasma ACTH and corticosterone levels. In rats, forced immobilisation and cold exposure decreased histamine levels and increased histamine turnover in the hypothalamus (21). An increase in the histamine concentration in the brain stems of the RD and K II groups may be due to the stress induced impairment of the release of histamine.

Even though REMS is effectively deprived by the flower pot technique, changes induced by this procedure are not necessarily a consequence only of selective REMSD. This experimental model induces stress due to isolation, immobilisation on the small platform and failing into the water (14, 17, 22). The water tank controls resided on an 11 cm diameter platform, which was large enough to permit REMS, as well as non-REM sleep and wakefulness (14). It was considered that the tank control group was an adequate control for the stressful conditions of the water tank.

Our results indicated that the activation of histaminergic neurons occurs during platform treatment, increasing the concentration of histamine in the brain stems of both RD and K II rats compared to K I rats. Further studies are needed to determine histamine syntheses enzyme activity or its rate of turnover in the hypothalamus of sleep deprived rats.

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