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Oral Mg^{2+} Supplementation Reverses Age-Related Neuroendocrine and Sleep EEG Changes in Humans

The process of normal aging is accompanied by changes in sleep-related endocrine activity. During aging, an increase in cortisol at its nadir and a decrease in renin and aldosterone concentration occur. In aged subjects, more time is spent awake and slow-wave sleep is reduced; there is a loss of sleep spindles and accordingly a loss of power in the sigma frequency range. Previous studies could show a close association between sleep architecture, especially slow-wave sleep, and activity in the glutamatergic and GABAergic system. Furthermore, recent studies could show that the natural N-methyl-D-aspartate (NMDA) antagonist and $GABA_A$ agonist Mg^{2+} seems to play a key role in the regulation of sleep and endocrine systems such as the HPA system and renin-angiotensin-aldosterone system (RAAS). Therefore, we examined the effect of Mg^{2+} in 12 elderly subjects (age range 60–80 years) on the sleep electroencephalogram (EEG) and nocturnal hormone secretion. A placebo-controlled, randomised cross-over design with two treatment intervals of 20 days duration separated by 2 weeks washout was used. Mg^{2+} was administered as effervescent tablets in a creeping dose of 10 mmol and 20 mmol each for 3 days followed by 30 mmol for 14 days. At the end of each interval, a sleep EEG was recorded from 11 p.m. to 7 a.m. after one accommodation night. Blood samples were taken every 30 min between 8 p.m. and 10 p.m. and every 20 min between 10 p.m. and 7 a.m. to estimate ACTH, cortisol, renin and aldosterone plasma concentrations, and every hour for arginine-vasopressin (AVP) and angiotensin II (ATII) plasma concentrations. Mg^{2+} led to a significant increase in slow wave sleep (16.5 ± 20.4 min *vs.* 10.1 ± 15.4 min, $p \leq 0.05$), delta power (47128.7 μV^2 ± 21417.7 μV^2 *vs.* 37862.1 μV^2 ± 23241.7 μV^2, $p \leq 0.05$) and sigma power (1923.0 μV^2 ± 1111.3 μV^2*vs.* 1541.0 μV^2 ± 1134.5 μV^2, $p \leq 0.05$). Renin increased (3.7 ± 2.3 ng/ml × min *vs.* 2.3 ± 1.0 ng/ml × min, $p < 0.05$) during the total night and aldosterone (3.6 ± 4.7 ng/ml × min *vs.* 1.1 ± 0.9 ng/ml × min, $p < 0.05$) in the second half of the night, whereas cortisol (8.3 ± 2.4 μg/ml × min *vs.* 11.8 ± 3.8 μg/ml × min, $p < 0.01$) decreased significantly and AVP by trend in the first part of the night. ACTH and ATII were not altered. Our results suggest that Mg^{2+} partially reverses sleep EEG and nocturnal neuroendocrine changes occurring during aging. The similarities of the effect of Mg^{2+} and that of the related electrolyte Li^+ furthermore supports the possible efficacy of Mg^{2+} as a mood stabilizer.

Introduction

The process of normal ageing is characterised by changes of CNS function leading to alteration of the sleep electroencephalogram (EEG) and hormone secretion. In the elderly, a decline in sleep continuity, a shortening of rapid eye movement (REM) latency, a decrease in slow wave sleep (SWS) and an increase in nocturnal wakefulness are detectable [9,28,65]. Beside these changes, a loss of the sleep dependent increase in spindle incidence and duration has been demonstrated in elderly [51].

In addition, ageing is accompanied by a disinhibition of HPA system activity in both experimental animals [35] and humans [38,84]. Another altered endocrine system in the course of ageing is the renin-angiotensin-aldosterone system (RAAS). An age-related decrease in plasma renin activity has been demonstrated in normotensive human subjects [22,60,71,83,86] accompanied by a tendency for a reduced aldosterone concentration [32]. Similarly, a reduced activity of the RAAS in experimental animals has been shown in aged compared to young animals [21,36].

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There is a close association between sleep architecture, especially SWS, and the activity of the glutamatergic and GABAergic system. An increase in SWS in rats was observed after intrathalamic administration of the NMDA antagonist DL-2-amino-5-phosphono-pentanoic acid (APV) [46] and after the intraperitoneal injection of NMDA antagonist MK-801 [16]. Also, the $GABA_A$ agonists THIP (gaboxadol) increased delta activity in rats [49,50] and SWS in young healthy controls [27] and in elderly humans [55]. An earlier study from our group on the effects of i.v. Mg^{2+} on the sleep EEG and nocturnal hormone secretion pointed to an NMDA-antagonistic and a GABA-agonistic efficacy of Mg^{2+} [59], an assumption well in line with preclinical studies [40,61,72,73,78].

Direct evidence for a disturbance of the glutamatergic neurotransmission in ageing was given by preclinical studies in mice demonstrating a reduced reuptake of glutamate in aged compared to younger animals [70]. The resulting glutamatergic overactivity might secondarily result in the age-related declines of NMDA receptors, as has been reported in rodents [20,80]. This is of interest since a decrease in NMDA binding sites has been correlated with a declining performance of memory-associated tasks [63].

Also, the changes in the sleep EEG occurring during ageing bear some similarities to those in Mg^{2+} deficiency. Mg^{2+} deficits lead to sleep disturbances with increased wakefulness at the expense of SWS [23] or a predominance of active waking and a decrease in total sleep time [64] in rats. On the other hand, there have also been reports on a sleep-stabilising effect of Mg^{2+}. Intravenous (i.v.) administration of Mg^{2+} before surgery led to a significantly better quality of sleep in a double blind trial on surgical patients [81].

Besides its modulatory influence on glutamatergic and GABAergic neurotransmission, Mg^{2+} also has an angiotensin II (ATII)-antagonistic effect [4,43], and is able to influence the HPA system [59]. The combination of these properties of Mg^{2+} influencing systems involved in the process of ageing and reports on its sleep stabilising effect led us to perform a study on the effect of oral Mg^{2+} supplementation on the sleep EEG and the nocturnal hormone secretion.

Materials and Methods

Subjects

Subjects consisted of 12 healthy volunteers (6 women, 6 men; age: range 60 – 80 years, mean ± SD: 68.1 ± 5.7 years) who entered the study after passing thorough psychiatric, physical and laboratory examinations. They had been drug-free for at least 4 weeks. Reasons for exclusion from the study were: psychiatric disorder history in the subjects or subject's family, including dementia or other cognitive impairment; recent stressful life events; substance abuse; a transmeridian flight during the last three months; shift work; medical illness; and aberrations in blood chemistry, waking EEG, or electrocardiogram. All subjects underwent a polysomnographic examination in the sleep laboratory before entering the study to exclude sleep disorder including sleep related respiratory disorders (such as sleep apnea) and sleep-related movement disorders (such as restless leg syndrome). Written informed consent was obtained from all subjects. The study was approved by the Ethics Committee of the *Bayerische Landesärztekammer* (Bavarian Regional Medical Association).

The study was performed in a double-blind, randomised, placebo-controlled crossover design with two treatment intervals of 20 days duration separated by 2 weeks washout. Mg^{2+} was administered as effervescent tablets (Biolectra ® Magnesium 240 forte produced by Hermes, Arzneimittel GmbH, Großhesselohe/Munich, each tablet containing 403.0 mg magnesiumoxid, as referred to in the text as 243 mg Mg^{2+}) in a creeping dose of 10 mmol (single administration in the morning) and 20 mmol (administration in the morning and at noon) each for 3 days followed by 30 mmol (administration in the morning, noon and evening) for 14 days. At the end of the two intervals, a sleep EEG was recorded after one night of adaptation to laboratory conditions, during which EEG electrodes were attached without recording an EEG. For the registration nights, the subjects arrived at the sleep laboratory at 7 p.m. At 7:30 p.m., an intravenous cannula for blood sampling was placed. Sleep EEG was recorded from 11p.m. until 7 a.m. next morning. Lights were switched of at 11 p.m. Sleep was not permitted before this time. The subjects were woken at 7 a.m. the next morning.

Blood samples were collected through an indwelling intravenous catheter connected to plastic tubing that ran through a soundproof lock into the adjacent room. This allowed repeated blood sampling without disturbing the subject's sleep. Blood samples were collected every 30 min between 8 p.m. and 10 p.m. and every 20 min between 10 p.m. and 7 a.m. for ACTH, cortisol, renin, and aldosterone. For AVP and ATII, blood samples were collected every hour. During the night, subjects were observed on a TV screen in the adjacent room.

Sleep EEG analysis

The sleep recordings were made from 11 p.m. to 7 a.m. and consisted of two EEGs (C3-A2, C4-A1; time constant 0.3 sec, low-pass filtering 70 Hz), vertical and horizontal electrooculograms (EOG), an electromyogram (EMG) and an electrocardiogram (ECG). The EEG signals were filtered (EEG: high pass 0.53 Hz, –3 dB; low pass 70 Hz, –3 dB; –12 dB octave, band-stop between 42 and 62 Hz, –3 dB) and transmitted by an optical fibre system to the polygraph (Schwartzer, ED 24). EEG signals were additionally sampled by an 8-bit analogue-to-digital converter at a sampling rate of 100 Hz using a personal computer and stored on disk for further spectral analysis. Sleep EEGs were rated visually according to standard criteria [67] by an experienced rater who was blind to the study protocol. Parameter used were: SOL – sleep onset latency (sleep onset defined as the first epoch of 30 seconds containing stages 2, 3, 4 or REM sleep) (min); SPT – sleep period time (interval from sleep onset until final awakening, including intermittent time spent awake) (min); TST – total sleep time (SPT – intermittent time spent awake) (min); time spent in each of the following sleep stages during TIB (min): Awake, non-REM stages 1 – 4, SWS – slow wave sleep (non-REM stages 3 and 4), REM; REM latency (interval from onset until the first epoch containing stage REM) (min); REM density (the average ratio of 3-second mini-epochs of REM sleep including REMs to the total number of 3-second mini-epochs of REM sleep). Spectral analysis was initially performed on the distinct frequency ranges delta (0.8 – 4.5 Hz), theta (4.5 – 8.0 Hz), alpha (8.0 – 11.8 Hz), sigma

(11.8 – 15.2 Hz) and beta (15.2 – 20.0 Hz) and thereafter using the single frequency bins. Because we were interested in the amount of sleep EEG power in the course of the night, we used an integrative method calculating the cumulative sum of each frequency bin during non-REM sleep in the course of the night.

Hormone measurements

Plasma ACTH, cortisol, AVP, renin, angiotensin II and aldosterone were measured by commercial radioimmunoassay. The intraassay variations were 5.6 – 6.9%, the interassay variations were 7.2 – 8.2%. Hormonal readings from each subject were analyzed in a separate assay.

Statistical analysis

Statistical analysis was performed separately for selected sleep parameters after visual analysis and for the two spectral ranges of interest, namely delta and sigma power. We used MANOVA with gender and sequence of treatment as factors. Calculations for endocrine variables were performed by computing the area under the curve (AUC) according to the trapezoid rule for distinct intervals as well as mean values. Hormone analysis (ACTH, cortisol, renin, and aldosterone) included the first (180 – 380 min) and second (380 – 660 min) part of the night, taking into account the fact that plasma cortisol and ACTH are low during the night, but begin to rise during the early morning hours. For ACTH, cortisol, renin, and aldosterone hormone analysis, we used MANOVA with gender and sequence of treatment as factors. Differences in AVP and ATII between placebo and Mg^{2+} subjects were assessed for significance in an exploratory manner with Wilcoxon's paired rank test for the AUC from 23.00 – 3.00, 3.00 – 7.00 separately. The analysis of blood electrolytes was established using the standard methods in the clinical laboratory of the Max Planck Institute of Psychiatry using the Roche/Hitachi 911 method. Differences were calculated in an exploratory manner with the Wilcoxon paired rank test. A value for $p \leq 0.05$ was considered significant; values at $p \leq 0.1$ were considered to reflect a trend for a significant difference. All data are expressed as mean ± standard deviation of mean.

Results

Subjects

Subjects consisted of 12 healthy volunteers (6 women, 6 men; age: range 60 – 80 years, mean ± SD: 68.1 ± 5.7 years). Besides the study medication, none of the subjects received any other drug during the study. There were no dropouts. All subjects complained about soft stool and one subject suffered a slight oedema of the extremities for the last week of active medication. Regarding side effects, no intervention was required, and no side effects were observed after the study had been concluded.

Comparing the placebo and verum condition, no significant change in plasma Mg^{2+} (0.82 ± 0.09 mmol/l after placebo *vs.* 0.86 ± 0.09 mmol/l after Mg^{2+}) was observed ($p > 0.1$). To take changes in water load into account, we calculated the Mg^{2+}/Na^{+} ratio. This ration increased by trend (0.0058 ± 0.0004 *vs.* 0.0061 ± 0.0007, $p < 0.1$). Sodium and potassium were unchanged (data not shown).

Sleep EEG analysis

A global effect of treatment for the selected sleep EEG parameters has been observed (Wilks, multivariate tests of significance F(6,3) = 12.32; significance of F = 0.032). The global effect is mainly caused by an increase of SWS as the univariate F-test could show ($p < 0.05$). No significant effect of the order of treatment or gender was observed for the conventional sleep EEG-parameter (Table **1**, Fig. **1**).

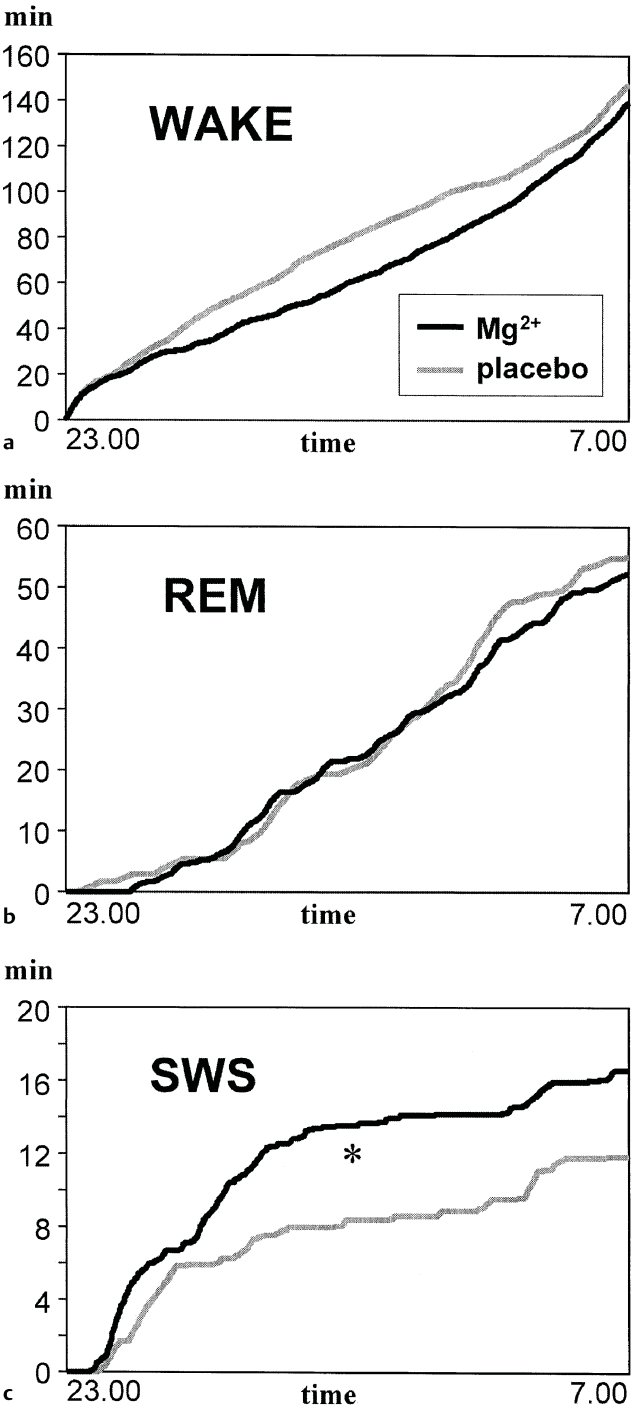

Fig. **1** The figure shows the mean of the cumulative duration the subjects stayed in the sleep stages wake (**a**), REM (**b**) and SWS (**c**). The amount of wakefulness was reduced insignificantly; REM sleep was not changed, whereas SWS was significantly increased with the major changes in the first part of the night. p = placebo condition, Mg^{2+} = magnesium supplementation.

Spectral analysis

As changes in the delta and sigma frequency range were of interest, we performed MANOVA on these two variables. We found a significant lower delta and sigma power in females compared to males (F (2,7) = 6.74, significance of F = 0.023) but no effect of gender or order of treatment on the treatment response. A significant global treatment effect was revealed, which was caused by a significant increase of delta (0.8 – 4.5 Hz) and sigma power (11.8 – 15.2 Hz); (univariate F–test, $p < 0.05$) (Table **2**, Fig. **2**). Qualitatively, no changes in the other spectral ranges occurred.

For technical reasons, not all subjects entered the analysis for the nocturnal hormone secretion. For the first part of the night, 11 subjects, and the second part of the night, 9 subjects entered the analysis of the nocturnal hormone secretion due to failures in blood samples.

Because of the relevant physiological differences in nocturnal hormone secretion in the first compared to the second part of the night, and also due to the different number of subjects entering the analysis for both time intervals, we independently performed distinct MANOVA for both intervals. The analysis was performed using the values ACTH, cortisol, renin and aldosterone with gender and rank in the sequence of the placebo and verum night as factors. For the first part of the night, we found a strong trend towards a global treatment effect resulting from a highly significant decrease in cortisol concentration and a significant increase in renin concentration. ACTH and aldosterone were unchanged. Separate analysis of AVP showed a trend to a decrease, but no change for ATII during this time interval (Table **3**; Fig. **3** and **4**). In the second part, no significant global effect occurred, but the renin and aldosterone concentration showed a significant increase. AVP and ATII were unchanged during this interval (univariate analysis, Table **3**; Fig. **3** and **4**).

Table **1** Effects of Mg^{2+} on the sleep EEG

Sleep parameter	*Placebo*	*Mg^{2+}*	*Treatment*
Global effect			p < 0.05 Univariate F-test
Total sleep time (min)	326.2 ± 59.4	336.1 ± 53.2	n.s
Sleep onset latency (min)	34.7 ± 34.0	19.3 ± 9.3	n.s
REM density (1/min)	5.7 ± 1.9	5.0 ± 1.6	n.s
REM latency (min)	112.6 ± 87.5	117.8 ± 80.9	n.s
Awake (SPT) (min)	97.9 ±5 4.2	103.4 ± 40.4	n.s
REM (TIB) (min)	54.7 ± 27.5	52.1 ± 21.9	n.s
Stage 2 (TIB) (min)	208.0 ± 51.0	219.8 ± 44.0	n.s
SWS (TIB) (min)	10.1 ± 15.4	16.5 ± 20.4	p < 0.05

Values are mean ± SD; SD: standard deviation of mean; REM: REM sleep duration; n.s: not significant; SPT: sleep period time; TIB: time in bed

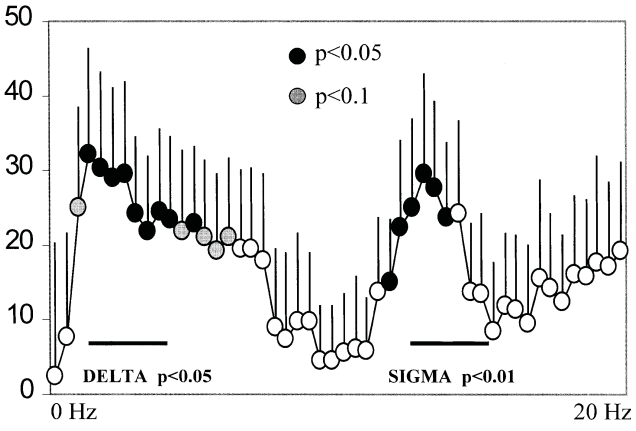

Fig. **2** The figure shows the percentage difference of the mean power values of non-REM sleep during the total night. Mg^{2+} led to a significant increase in the delta and sigma frequency range. Black circles show a significant level of $p < 0.05$; grey circles show a trend with $p < 0.1$.

Table **2** Spectral analysis for the distinct frequency ranges (μV^2)

	Placebo	*Mg^{2+}*	*Treatment*
Global effect (Delta /Sigma)			p < 0.05
Delta	37 862.1 ± 23 241.7	47 128.7 ± 21 417.7	p < 0.05
Theta	5 004.5 ± 3 629.1	5 926.2 ± 3 278.2	n.s.
Alpha	3 553.1 ± 2 839.4	3 940.2 ± 2 506.2	n.s.
Sigma	1 541.0 ± 1 134.5	1 923.0 ± 1 111.3	p < 0.05
Beta	726.9 ± 446.5	883.4 ± 502.5	n.s.

Values are mean ± SD; n.s: not significant; SD: standard deviation of mean

Discussion

The major effects of oral Mg^{2+} supplementation in elderly are 1.) an increase of SWS, 2.) an increase in delta power and sigma

Table **3** Effects of Mg^{2+} on nocturnal hormone secretion

Hormone	*Placebo*	*Mg^{2+}*	*Treatment*
1st part (n = 11)			global: F (4,4) = 4.5; p < 0.09
			Univariate F-Test
AVP *	0.1 ± 0.07	0.1 ± 0.03	n.s.; (p < 0.1)
ACTH	2.6 ± 0.8	2.4 ± 0.6	F = 0.8; n.s.
Cortisol	11.8 ± 3.8	8.3 ± 2.4	F = 11.2; p < 0.02
Renin	1.0 ± 0.4	1.6 ± 0.9	F = 7.4; p < 0.05
Angiotensin II *	2.8 ± 1.1	2.4 ± 0.7	n.s.
Aldosterone	1.1 ± 0.9	3.6 ± 4.7	F = 2.8; n.s.
2nd part (n = 9)			global: F (4,2) = 1.4; n.s.
			Univariate F-Test
AVP *	0.3 ± 0.1	0.2 ± 0.09	n.s.
ACTH	6.3 ± 3.4	8.1 ± 2.8	F = 0.0; n.s.
Cortisol	33.6 ± 12.9	35.5 ± 7.9	F = 0.5; n.s.
Renin	1.4 ± 0.7	2.1 ± 1.3	F = 7.8; p < 0.05
Angiotensin II *	5.3 ± 1.9	4.9 ± 1.3	n.s.
Aldosterone	2.9 ± 1.7	10.4 ± 7.6	F = 9.9; p < 0.05

Values represent the area under the curve for the time from 23.00 h to 2.20 h (first part) and from 2.20 h to 7.00 h (second part). Results are given as ng/ml x min for ACTH, AVP, renin, angiotensin and aldosterone, µg/ml x min for cortisol. * Blood samples were taken every 60 min for AVP and angiotensin and did not enter the MANOVA; n.s.: not significant

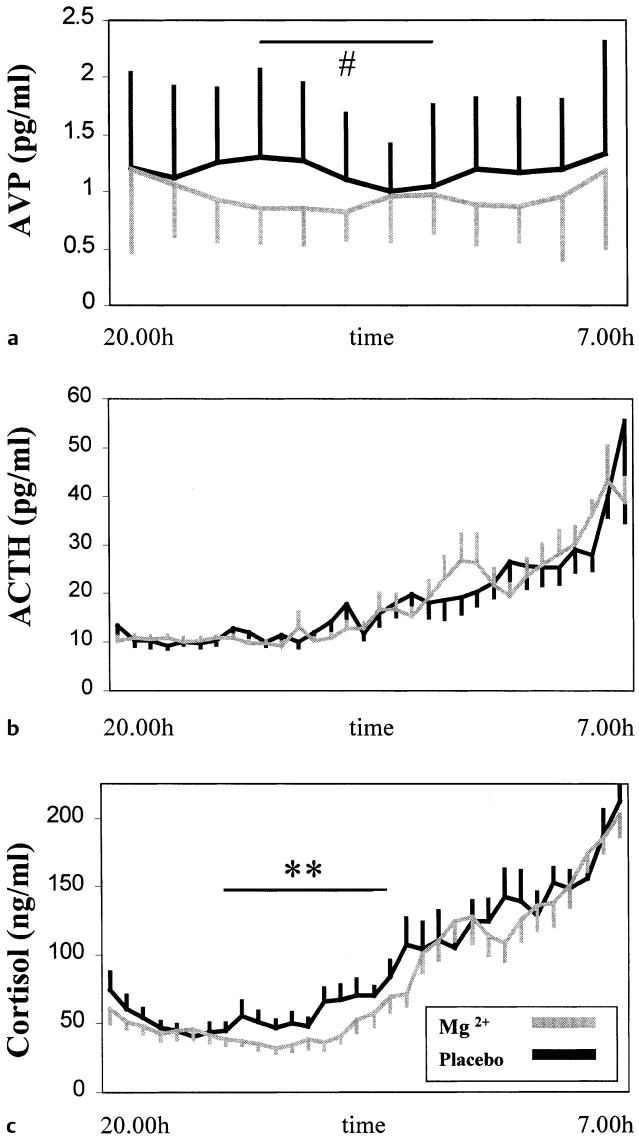

Fig. **3** Time course of nocturnal hormone secretion under placebo and Mg^{2+}. AVP (**a**); ACTH (**b**); cortisol (**c**); Figure (**a**) showed a trend towards a decrease of AVP ($p < 0.1$) throughout the night. No changes in ACTH (**b**) secretion occurred. Cortisol (**c**) is significant reduced after Mg^{2+} in the first half of the night ($p < 0.01$). Grey line: Mg^{2+}; Black line: placebo; # $p < 0.1$; ** $p < 0.01$.

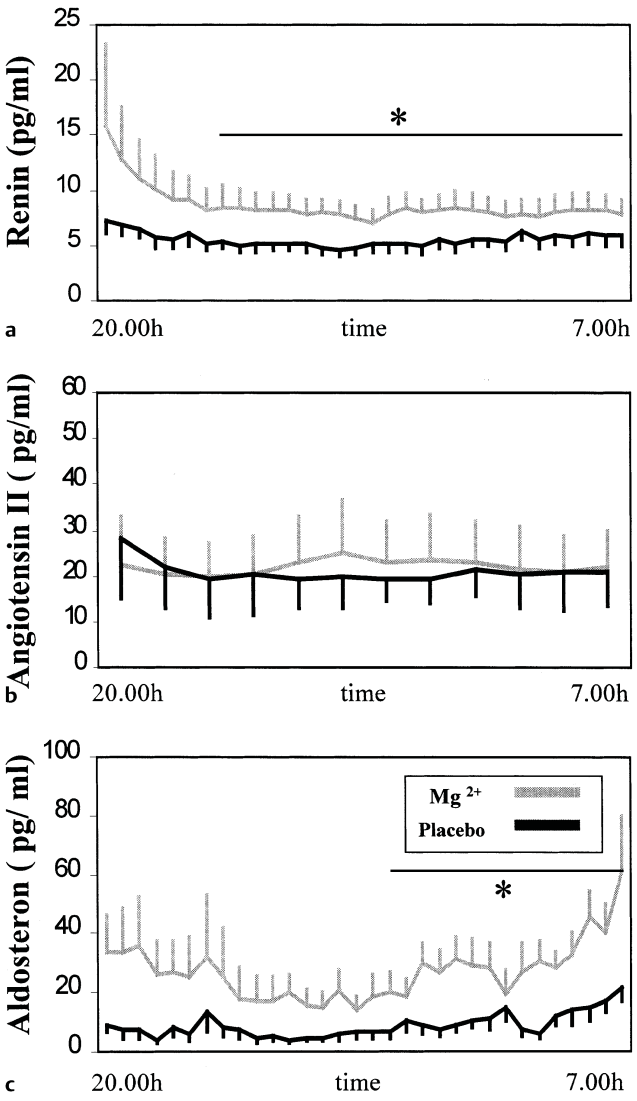

Fig. **4** Time course of nocturnal hormone secretion under placebo and Mg^{2+}. Renin (**a**); angiotensin II (**b**); aldosterone (**c**); Renin (**a**) and aldosterone (**c**) were significant increased after Mg^{2+} throughout the night ($p < 0.05$). No changes in angiotensin II (**b**) secretion occurred. Grey line: Mg^{2+}; Black line: placebo; * $p < 0.05$.

power during non-REM sleep, 3.) a decrease in the concentration of cortisol during the first part of the night, 4.) an increase of the renin concentration throughout and of the aldosterone concentration in the second part of the night, 5.) a trend to a decrease of AVP in the first part, and 6.) no alteration of ACTH and ATII in any part of the night. The endocrine changes reported here have, however, only exploratory character, as MANOVA could not reveal significant global effects for the endocrine parameters as a whole, which is due to the design with multiple testing. However, the results seem highly relevant for the understanding of the action of Mg^{2+}, especially in the context of the sleep EEG data.

In the present study, we only found a trend towards an increase in plasma Mg^{2+} concentration, measured using the Roche/Hitachi 911 method, when related to plasma Na^{+}. For the actions observed in the sleep-EEG, the free intracerebral Mg^{2+} concentration has been suggested to be relevant. On the one hand it has been shown that Mg^{2+} enters the brain after an iv bolus injection [33]. On the other hand, Mg^{2+} is actively transported *via* the blood-brain barrier by an energy-dependent process, leading to a higher intracerebral concentration than that of the plasma [8,57]. Therefore, plasma Mg^{2+} concentrations cannot be used to predict the changes in the CSF concentration [33]. As active transport systems often depend on Mg^{2+} [57], it might well be that Mg^{2+} administration activates its own transport *via* the blood-brain barrier. The possibility that the described effects are secondary to the endocrine changes after Mg^{2+} will be discussed later.

Our results on the effect of Mg^{2+} at the sleep EEG agree with the observation of a decrease of SWS in rats with Mg^{2+} deficiency [23] and reports of a sleep-stabilising effect of Mg^{2+} in patients before surgery [81]. A highly positive correlation with the length

of SWS and the levels of Mg^{2+} in specific brain areas was observed in mice [18]. Furthermore, i.v. Mg^{2+} administration in healthy young subjects led to an increase of EEG power in the sigma frequency range of non-REM sleep, which is also in line with our present findings [59]. On the other hand, no change in SWS could be observed in our earlier examination, pointing to a difference between acute and chronic administration. The subjects in both studies had no clinical signs of Mg^{2+} deficiency. A subclinical Mg^{2+} deficiency, however, could not be ruled out. The findings also differ from the effect of Mg^{2+} on the sleep of patients with restless leg syndrome [41], as mainly amelioration in the sleep continuity, but no change in SWS was observed in this study. We, of course, explicitly screened for restless leg syndrome to exclude this population.

Ageing is associated with decreased sleep continuity, SWS and a loss of the sleep-dependent increase in spindle incidence and duration and accordingly power in the sigma frequency range which predominantly includes sleep spindles [51]. In addition, there is a reduced activity of RAAS and increased activity of the HPA system with increasing age [9, 38, 84]. Mg^{2+} therefore seems to be able to reverse age related changes in the sleep EEG.

What are possible mechanisms for the pharmacological action of Mg^{2+}? The voltage-dependent blockage of extracellular Mg^{2+} on NMDA-gated channel has been thoroughly characterised [61, 72, 78]. As the selective NMDA receptor antagonist APV causes an increase of SWS in cats [46], the non-competitive NMDA channel blockade by MK 801 intensely stimulates the power in the delta-frequency range in non-REM sleep [17] and the NMDA receptor antagonist amantadine leads to an increase in spindle power in patient with Parkinson's disease [66]. The effect demonstrated in this study resembles those with NMDA antagonists and might point to NMDA antagonistic effect of Mg^{2+} on sleep.

Besides the glutamatergic system, the GABAergic system is influenced by Mg^{2+}. Response to the $GABA_A$ agonist muscimol is enhanced by Mg^{2+} in rat cerebral cortical synaptoneurosomes [73]. Additionally, spontaneous paroxysmal discharges in rat cerebral cortex induced by omitting Mg^{2+} from the superfusion medium can be dampened by the $GABA_A$ agonist muscimol [40]. Regarding sleep, the $GABA_A$ agonists THIP (gaboxadol) increased delta activity in rats [49, 50] and SWS in humans [27]. Also, GABA/benzodiazepine agonists [1] increase the occurrence of sleep spindles. Therefore, the changes in the sleep EEG observed here might also be a result of a GABAergic action of Mg^{2+}.

Besides NMDA antagonistic and GABA agonistic properties, Mg^{2+} also has endocrine effects such as an ATII-antagonistic action [4, 43] and a dampening effect on HPA-system activity [59]. In the present study, a decrease of cortisol but not ACTH occurred after chronic oral administration of Mg^{2+}. As a decrease in cortisol should lead to an increase of ACTH *via* an attenuated feedback, which we did not find here, the pattern of the present findings must be attributed to a decrease in central (hypothalamic) HPA system activity. NMDA antagonists also decrease HPA-system activity [26, 44, 45, 47]. Together these effects are in line with an NMDA antagonistic property of Mg^{2+}. Additionally, $GABA_A$ agonists increase delta EEG power and SWS [27] and decrease HPA-system activity [56], additionally pointing to a possible influence on GABAergic neurotransmission.

Concerning the RAAS, Mg^{2+} blocks the increase of aldosterone secretion stimulated by ATII in the rat and decreases plasma aldosterone concentration while increasing plasma renin activity [43]. Also, Mg^{2+} decreases the AT II induced aldosterone production of adrenal cells [3], whereas Mg^{2+} deprivation leads to an enhancement of the increase of intracellular Ca^{2+} response to ATII and AVP in vascular muscle cells [88]. In our study, we observed an increase of renin and aldosterone secretion after Mg^{2+} administration. The increase of renin is in line with an ATII-antagonistic effect of Mg^{2+} *via* an attenuated negative feedback of ATII on renin release [5, 74, 77]. The observed increase of aldosterone in our study can not be easily explained. Chronic administration of aldosterone, however, leads to an enhancement of urinary excretion of Mg^{2+} [42]. In that respect, an increase of aldosterone might make sense from a homeostatic standpoint, regulating Mg^{2+} down to its physiological level in the face of Mg^{2+} loading.

Endocrine changes as cause of sleep EEG changes?

During the first part of the night, the increase in SWS is accompanied by a decrease in cortisol, that is, during an interval when the physiological cortisol concentration is low. During the second part, cortisol is unchanged. The decreased concentration of cortisol during a limited interval should not result in an increase in SWS, as an infusion of cortisol in young [30] and elderly [10] subjects lead to an increase in SWS. On the other hand, during SWS, HPA-system activity is reduced [2, 6], pointing to a common regulatory mechanism for both phenomena [75].

Could the effects on sleep be related to changes in the RAAS? No influence of mineralocorticoid aldosterone [14] or the mineralocorticoid receptor (MR) antagonist spironolactone [76] on sleep has been reported. However, the MR antagonist canreonoate leads to a decrease in SWS in humans [12] and furthermore to an attenuation of the SWS related cortisol suppression [13]. Also, sub-chronic administration of the MR antagonist to elderly human subjects leads to a hypercortisolism [37]. Therefore, one could suggest that the increase in aldosterone might be the cause for the decline in HPA-system activity and the increase in SWS. As the increase in aldosterone is only significant in the second part of the night, this interpretation cannot be directly supported, but also cannot be ruled out due to lack of knowledge about the changes of aldosterone and their influence on sleep. As SWS is associated with an increase of plasma renin activity [53], a common regulatory mechanism of Mg^{2+} on SWS and the RAAS is probable. ATII stimulates the secretion of ACTH by a mechanism that can be blocked by antibodies to CRH and drugs known to decrease CRH secretion [34]. Apart form that, icv ATII increases CRH mRNA in the rat hypothalamus [79] and stimulates the secretion of AVP. Both effects could be blocked by the competitive ATII inhibitor saralasin [15]. As Mg^{2+} also has ATII antagonistic effects [3, 43], the decreased HPA activity found in our study might be related to this property. Moreover, as glutamate leads to an increase of angiotensin receptor mRNA level that can be suppressed by the NMDA antagonist MK801 in cultured rat cortical cells [54], the ATII antagonistic effects of Mg^{2+} could be related to its NMDA antagonistic properties.

Regardless of the possible pharmacological action of Mg^{2+}, the sleep EEG is an indicator of CNS function. It is interesting in this context that a severe reduction of sleep spindles accompanies the development of dementia [68], so sleep spindles could be a

marker for cognitive functioning. The competitive NMDA-receptor antagonist, amantadine, which acts synergistically with Mg^{2+} on hippocampal neurones, increases sleep spindles in patient with Parkinson's disease [66]; studies have shown an improvement of cognitive function in patients with senile dementia [58] and multiple sclerosis [69] under the treatment with this substance. The closely related substance, memantine, has been shown to act synergistically with Mg^{2+} on long term potentiation (LTP), a model of cognitive function [29]. Good evidence exists that memantine leads to an improvement of cognitive function in dementia [24]. An additional argument for an interaction of Mg^{2+} and cognitive functioning is the finding of a significant correlation between cognitive impairment and the Mg/K ratio in lymphocytes in subjects with and without dementia [11].

Ageing is associated with changes of non-REM sleep and HPA system activity similar to those seen in depression [9,38,39,52,84,75]. Additionally, aging is accompanied by an increased risk of depression [85,87]. In that respect, aging might partially be a model of depression. Consequently, our finding presented here could have implications for the treatment of depression. In fact, Mg^{2+} seems to have mood-stabilising properties [19,25,62]. Interestingly, continued treatment with lithium, the most well-established mood stabilising medication, results in raised plasma renin and aldosterone concentrations, which are positively correlated to serum magnesium [82]. This activation of the RAAS by lithium resembles the effects of Mg^{2+} supplementation we described here. Additionally, lithium leads to similar changes in sleep we observed with Mg^{2+}, especially to an increase in SWS [7,31,48]. Our results therefore support the assumption that Mg^{2+} may be a useful supplementary therapy for the clinical management of affective disorder.

In summary, we have demonstrated that the oral administration of Mg^{2+} partially reverses changes in sleep EEG and the endocrine system that occur during ageing. Mg^{2+} leads to an increase of SWS and delta and sigma power, an increase in renin and aldosterone secretion and a decrease in cortisol and by trend in AVP. As possible mechanisms of Mg^{2+}, we have discussed an NMDA-antagonistic, GABA-agonistic or ATII-antagonist effect. The present study presents the possibility that Mg^{2+} might be useful in ageing-related disorders and, because the similarity effects to those of lithium, further points to its possible use as a mood stabilizer. Further studies should be performed to test these hypotheses directly.

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References

[1] Aeschbach D, Dijk DJ, Trachsel L, Brunner DP, Borbely AA. Dynamics of slow-wave activity and spindle frequency activity in the human sleep EEG: effect of midazolam and zopiclone. Neuropsychopharmacology 1994; 11: 237 – 244

[2] Antonijevic IA, Murck H, Frieboes RM, Schier T, Holsboer F, Steiger A. Hyporesponsiveness of the pituitary to CRH during slow wave sleep is not mimicked by systemic GHRH. Neuroendocrinology 1999; 69: 88 – 96

[3] Atarashi K, Matsuoka H, Takagi M, Sugimoto T. Magnesium ion: a possible physiological regulator of aldosterone production. Life Sci. 1989; 44: 1483 – 1489

[4] Atarashi K, Takagi M, Matsuoka H, Sugimoto T. Effects of magnesium on changes in blood pressure and plasma aldosterone induced by angiotensin II. Am.J.Hypertens. 1990; 3: 488 – 490

[5] Bader M, Ganten D. Regulation of renin:new evidence from cultured cells and genetically modified mice. Journal of Moleculare Medicine 2000; 78: 130 – 139

[6] Bierwolf C, Struve K, Marshall L, Born J, Fehm HL. Slow wave sleep drives inhibition of pituitary-adrenal secretion in humans. J.Neuroendocrinol. 1997; 9: 479 – 484

[7] Billiard M. Lithium carbonate: effects on sleep patterns of normal and depressed subjects and its use in sleep-wake pathology. Pharmacopsychiatry 1987; 20: 195 – 196

[8] Bito LZ. Blood-brain barrier: evidence for active cation transport between blood and the extracellular fluid of brain. Science 1968; 165: 81 – 83

[9] Bliwise DL. Sleep in normal aging and dementia. Sleep 1993; 16: 40 – 81

[10] Bohlhalter S, Murck H, Holsboer F, Steiger A. Cortisol enhances non-REM sleep and growth hormone secretion in elderly subjects. Neurobiol.Aging 1997; 18: 423 – 429

[11] Borella P, Giardino A, Neri M, Andermarker E. Magnesium and potassium status in elderly subjects with and without dementia of the Alzheimer type. Magnesium Research 1990; 3: 283 – 289

[12] Born J, De Kloet ER, Wenz H, Kern W, Fehm HL. Gluco- and antimineralocorticoid effects on human sleep: a role of central corticosteroid receptors. Am.J.Physiol. 1991; 260: E183 – 188

[13] Born J, Steinbach D, Dodt C, Fehm HL. Blocking of central nervous mineralocorticoid receptors counteracts inhibition of pituitary-adrenal activity in human sleep. J.Clin.Endocrinol.Metab. 1997; 82: 1106 – 1110

[14] Born J, Zwick A, Roth G, Fehm-Wolfsdorf G, Fehm HL. Differential effects of hydrocortisone, fluocortolone, and aldosterone on nocturnal sleep in humans. Acta Endocrinol.(Copenh.) 1987; 116: 129 – 137

[15] Brooks VL, Malvin RL. An intracerebral, physiological role for angiotensin: effects of central blockade. Fed.Proc. 1979; 38: 2272 – 2275

[16] Campbell IG, Feinberg I. Noncompetitive NMDA channel blockade during waking intensely stimulates NREM delta. J. Pharmacol. Exp. Ther. 1996b; 276: 737 – 742

[17] Campbell IG, Feinberg I. NREM delta stimulation following MK-801 is a response of sleep systems. J.Neurophysiol. 1996a; 76: 3714 – 3720

[18] Chollet D, Franken P, Raffin Y, Malafosse A, Widmer J, Tafti M. Blood and brain magnesium in inbred mice and their correlation with sleep quality. Am.J.Physiol.-Reg.I. 2000; 279: R2173 – 2178

[19] Chouinard G, Beauclair L, Geiser R, Etienne P. A pilot study of magnesium aspartate hydrochloride (Magnesiocard) as a mood stabilizer for rapid cycling bipolar affective disorder patients. Prog.Neuropsychopharmacol.Biol.Psychiatry 1990; 14: 171 – 180

[20] Clark AS, Magnusson KR, Cotman CW. In vitro autoradiography of hippocampal excitatory amino acid binding in aged Fischer 344 rats: relationship to performance on the Morris water maze. Behav.Neurosci. 1992; 106: 324 – 335

[21] Corman B, Michel JB. Renin-angiotensin system, converting-enzyme inhibition and kidney function in aging female rats. Am.J.Physiol. 1986; 251: R450 – 455

[22] Crane MG, Harris JJ. Effect of aging on renin activity and aldosterone excretion. J.Lab.Clin.Med. 1976; 87: 947 – 959

[23] Depoortere H, Francon D, Llopis J. Effects of a magnesium-deficient diet on sleep organization in rats. Neuropsychobiology 1993; 27: 237 – 245

[24] Ditzler K. Efficacy and tolerability of memantine in patients with dementia syndrome. A double-blind, placebo controlled trial. Arzneimittelforschung 1991; 41: 773 – 780

[25] Facchinetti F, Borella P, Sances G, Fioroni L, Nappi RE, Genazzani AR. Oral magnesium successfully relieves premenstrual mood changes. Obstet.Gynecol. 1991; 78: 177 – 181

[26] Farah JM Jr., Rao TS, Mick SJ, Coyne KE, Iyengar S. N-methyl-D-aspartate treatment increases circulating adrenocorticotropin and luteinizing hormone in the rat. Endocrinology 1991; 128: 1875 – 1880

[27] Faulhaber J, Steiger A, Lancel M. The $GABA_A$ agonist THIP produces slow wave sleep and reduces spindling activity in Non-REM sleep in humans. Psychopharmacology 1997; 130: 285 – 291

[28] Feinberg I. Changes in sleep cycle patterns with age. J.Psychiatr.Res. 1974; 10: 283 – 306

[29] Frankiewicz T, Parsons CG. Memantine restores long term potentiation impaired by tonic N-methyl-D-aspartate (NMDA) receptor activation following reduction of Mg2+ in hippocampal slices. Neuropharmacology 1999; 38: 1253 – 1259

[30] Friess E, Tagaya H, Grethe C, Trachsel L, Holsboer F. Cortisol infusion increases EEG slow wave activity within non-REM sleep in humans. J.Sleep Res. 1996; 5 (Suppl. 1): 69

[31] Friston KJ, Sharpley AL, Solomon RA, Cowen PJ. Lithium increases slow wave sleep: possible mediation by brain 5-HT2 receptors? Psychopharmacology 1989; 98: 139 – 140

[32] Frolkis VV, Verkhratsky NS, Magdich LV. Regulation of aldosterone secretion in old rats. Gerontology 1985; 31: 84 – 94

[33] Fuchs-Buder T, Tramer MR, Tassonyi E. Cerebrospinal fluid passage of intravenous magnesium sulfate in neurosurgical patients. J Neurosurg Anesthesiol 1997; 9: 324 – 328

[34] Ganong WF. Blood, pituitary, and brain renin-angiotensin systems and regulation of secretion of anterior pituitary gland. Front.Neuroendocrinol. 1993; 14: 233 – 249

[35] Hatzinger M, Reul JM, Landgraf R, Holsboer F, Neumann I. Combined dexamethasone/CRH test in rats: hypothalamo-pituitary- adrenocortical system alterations in aging. Neuroendocrinology 1996; 64: 349 – 356

[36] Hayashi M, Saruta T, Nakamura R, Kitajima W, Kato E. Effect of aging on single nephron renin content in rats. Renal Physiology 1981; 4: 17 – 21

[37] Heuser I, Deuschle M, Weber A, Kniest A, Ziegler C, eber B, olla M. The role of mineralocorticoid receptors in the circadian activity of the human hypothalamus-pituitary-adrenal system. Neurobiol.Aging 2000; 21: 585 – 589

[38] Heuser IJ, Gotthardt U, Schweiger U, Schmider J, Lammers CH, Dettling M, Holsboer F. Age-associated changes of pituitary-adrenocortical hormone regulation in humans: importance of gender. Neurobiol. Aging 1994; 15: 227 – 231

[39] Hoch CC, Dew MA, Reynolds CF3, Monk TH, Buysse DJ, Houck PR, Machen MA, Kupfer DJ. A longitudinal study of laboratory- and diary-based sleep measures in healthy "old old" and "young old" volunteers. Sleep 1994; 17: 489 – 496

[40] Horne AL, Harrison NL, Turner JP, Simmonds MA. Spontaneous paroxysmal activity induced by zero magnesium and bicuculline: suppression by NMDA antagonists and GABA mimetics. Eur.J.Pharmacol. 1986; 122: 231 – 238

[41] Hornyak M, Voderholzer U, Hohagen F, Berger M, Riemann D. Magnesium therapy for periodic leg movements-related insomnia and restless legs syndrome: an open pilot study. Sleep 1998; 21: 501 – 505

[42] Horton R, Biglieri EG. Effect of Aldosterone on the Metabolism of Magnesium. J.Clin.Endocrinol.Metab. 1962; 22: 1187 – 1192

[43] Ichihara A, Suzuki H, Saruta T. Effects of magnesium on the renin-angiotensin-aldosterone system in human subjects. J.Lab.Clin.Med. 1993; 122: 432 – 440

[44] Jezova D, Oliver C, Jurcovicova J. Stimulation of adrenocorticotropin but not prolactin and catecholamine release by N-methyl-aspartic acid. Neuroendocrinology 1991; 54: 488 – 492

[45] Jezova D, Tokarev D, Rusnak M. Endogenous excitatory amino acids are involved in stress-induced adrenocorticotropin and catecholamine release. Neuroendocrinology 1995; 62: 326 – 332

[46] Juhasz G, Kekesi K, Emri Z, Soltesz I, Crunelli V. Sleep-promoting action of excitatory amino acid antagonists: a different role for thalamic NMDA and non-NMDA receptors. Neurosci.Lett. 1990; 114: 333 – 338

[47] Kniest A, Wiesenberg C, Weber B, Colla M, Heuser IJ, Deuschle M. The Glutamate Antagonist Riluzole and Its Effects upon Basal and Stress-Induced Activity of the Human Hypothalamus- Pituitary-Adrenocortical System in Elderly Subjects. Neuropsychobiology 2001; 43: 91 – 95

[48] Kupfer DJ, Reynolds CF, Weiss BL, Foster FG. Lithium carbonate and sleep in affective disorders. Further considerations. Arch Gen Psychiatry 1974; 30: 79 – 84

[49] Lancel M. The GABA(A) agonist THIP increases non-REM sleep and enhances non-REM sleep-specific delta activity in the rat during the dark period. Sleep 1997; 20: 1099 – 1104

[50] Lancel M, Faulhaber J. The $GABA_A$ agonist THIP (gaboxadol) increases non-REM sleep and enhances delta activity in the rat. NeuroReport 1996; 7: 2241 – 2245

[51] Landolt HP, Dijk DJ, Achermann P, Borbély AA. Effect of age on the sleep EEG: slow-wave activity and spindle frequency activity in young and middle-aged men. Brain Res. 1996; 738: 205 – 212

[52] Lauer C, Riemann D, Wiegand M, Berger M. From early to late adulthood. Changes in EEG sleep of depressed patients and healthy volunteers. Biol.Psychiatry 1991; 29: 979 – 993

[53] Luthringer R, Brandenberger G, Schaltenbrand N, Muller G, Spiegel K, Macher JP, Muzet A, Follenius M. Slow wave electroencephalic activity parallels renin oscillations during sleep in humans. Electroencephalogr.Clin.Neurophysiol. 1995; 95: 318 – 322

[54] Makino I, Shibata K, Shibaguchi H, Niwa M, Katsuragi T, Furukawa T. The increase in angiotensin type-2 receptor mRNA level by glutamate stimulation in cultured rat cortical cells. Brain Res. 1998; 804: 296 – 305

[55] Mathias S, Wetter TC, Steiger A, Lancel M. The GABA uptake inhibitor tiagabine promotes slow wave sleep in normal elderly subjects. Neurobiol.Aging 2001; 22(2): 247 – 253

[56] Miguez I, Aldegunde MA. Effect of gamma-aminobutyric acid on corticosterone secretion: involvement of the noradrenergic system. Life Sci. 1990; 46: 875 – 880

[57] Morris ME. Brain and CSF magnesium concentrations during magnesium deficit in animals and humans: neurological symptoms. Magnes Res 1992; 5: 303 – 313

[58] Muller HF, Dastoor DP, Klingner A, Cole M, Boillat J. Amantadine in senile dementia: electroencephalographic and clinical effects. J.Am.Geriatr.Soc. 1979; 27: 9 – 16

[59] Murck H, Steiger A. Mg^{2+} reduces ACTH secretion and enhances spindle power without changing delta power during sleep in men – possible therapeutical implications. Psychopharmacology 1998; 137: 247 – 252

[60] Noth RH, Lassman MN, Tan SY, Fernandez-Cruz AJ, Mulrow PJ. Age and the renin-aldosterone system. Arch.Intern.Med. 1977; 137: 1414 – 1417

[61] Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A. Magnesium gates glutamate-activated channels in mouse central neurones. Nature 1984; 307: 462 – 465

[62] Pavlinac D, Langer R, Lenhard L, Deftos L. Magnesium in affective disorders. Biol.Psychiatry 1979; 14: 657 – 661

[63] Pelleymounter MA, Beatty G, Gallagher M. Hippocampal 3H-CPP binding and spatial learning deficits in aged rats. Psychobiology 18 (3) 1929; 1990: 298 – 304

[64] Poenaru S, Rouhani S, Durlach J, Aymard N, Belkahla F, Rayssiguier Y, Iovino M. Vigilance states and cerebral monoamine metabolism in experimental magnesium deficiency. Magnesium 1984; 3: 145 – 151

[65] Prinz PN, Peskind ER, Vitaliano PP, Raskind MA, Eisdorfer C, Zemcuznikov N, Gerber CJ. Changes in the sleep and waking EEGs of nondemented and demented elderly subjects. J.Am.Geriatr.Soc. 1982; 30: 86 – 93

[66] Puca FM, Bricolo A, Turella G. Effect of L-dopa or amantadine therapy on sleep spindles in Parkinsonism. Electroencephalogr.Clin.Neurophysiol. 1973; 35: 327 – 330

[67] Rechtschaffen A, Kales A. A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects. US Department of Health, Education & Welfare, Neurological Information Network, Bethesda, MD: 1968

[68] Reynolds CF3, Kupfer DJ, Taska LS, Hoch CC, Spiker DG, Sewitch DE, Zimmer B, Marin RS, Nelson JP, Martin Det al. EEG sleep in elderly depressed, demented, and healthy subjects. Biol.Psychiatry 1985; 20: 431 – 442

[69] Sailer M, Heinze HJ, Schoenfeld MA, Hauser U, Smid HG. Amantadine influences cognitive processing in patients with multiple sclerosis. Pharmacopsychiatry 2000; 33: 28 – 37

[70] Saransaari P, Oja SS. Age-related changes in the uptake and release of glutamate and aspartate in the mouse brain. Mech.Ageing Develop. 1995; 81: 61 – 71

[71] Saruta T, Suzuki A, Hayashi M, Yasui T, Eguchi T, Kato E. Mechanism of age-related changes in renin and adrenocortical steroids. J.Am.Geriatr.Soc. 1980; 28: 210 – 214

[72] Schneiderman JH, Arnold D, Advani A. Different changes in spontaneous field potential oscillations precede epileptiform bursting in hippocampal slices perfused with penicillin or reduced magnesium. Brain Res. 1990; 533: 275 – 285

[73] Schwartz RD, Wagner JP, Yu X, Martin D. Bidirectional modulation of GABA-gated chloride channels by divalent cations: inhibition by Ca^{2+} and enhancement by Mg^{2+}. J.Neurochem. 1994; 62: 916 – 922

[74] Shricker K, Holmer S, Kramer BK, Riegger GA, Kurtz A. The role of angiotensin II in the feedback control of renin gene expression. Pflugers Archiv – European Journal of Physiology 1997; 434: 166 – 172

[75] Steiger A. Sleep and the hypothalamo-pituitary-adrenocortical-system. Sleep Medicine Reviews, 2001; in press
[76] Steiger A, Rupprecht R, Spengler D, Guldner J, Hemmeter U, Rothe B, Damm K, Holsboer F. Functional properties of deoxycorticosterone and spironolactone: molecular characterization and effects on sleep-endocrine activity. J.Psychiatr.Res. 1993; 27: 275 – 284
[77] Stella A, Macchi A, Genovesi S, Centonza L, Zanchetti A. Angiotensin converting enzyme inhibition and renin release from the kidney. Journal of Hypertension – Supplement 1989; 7: S21 – 25
[78] Stevens DR, McCarley RW, Greene RW. Excitatory amino acid-mediated responses and synaptic potentials in medial pontine reticular formation neurons of the rat in vitro. J.Neurosci. 1992; 12: 4188 – 4194
[79] Sumitomo T, Suda T, Nakano Y, Tozawa F, Yamada M, Demura H. Angiotensin II increases the corticotropin-releasing factor messenger ribonucleic acid level in the rat hypothalamus. Endocrinology 1991; 128: 2248 – 2252
[80] Tamaru M, Yoneda Y, Ogita K, Shimizu J, Nagata Y. Age-related decreases of the N-methyl-D-aspartate receptor complex in the rat cerebral cortex and hippocampus. Brain Res. 1991; 542: 83 – 90
[81] Tramer MR, Schneider J, Marti RA, Rifat K. Role of magnesium sulfate in postoperative analgesia. Anesthesiology 1996; 84: 340 – 347
[82] Transbol I, Christiansen C, Baastrup PC, Nielsen MD, Giese J. Endocrine effects of lithium. III. Hypermagnesaemia and activation of the renin-aldosterone system. Acta Endocrinol. 1978; 88: 619 – 624
[83] Tsunoda K, Abe K, Goto T, Yasujima M, Sato M, Omata K, Seino M, Yoshinaga K. Effect of age on the renin-angiotensin-aldosterone system in normal subjects: simultaneous measurement of active and inactive renin, renin substrate, and aldosterone in plasma. J. Clin. Endocrinol. Metab. 1986; 62: 384 – 389
[84] Van Coevorden A, Mockel J, Laurent E, Kerkhofs M, L'Hermite-Balériaux M, Decoster C, Neve P, Van Cauter E. Neuroendocrine rhythms and sleep in aging men. Am.J.Physiol. 1991; 260: E651 – 661
[85] Veith RC, Raskind MA. The neurobiology of aging: does it predispose to depression? Neurobiol.Aging 1988; 9: 101 – 117
[86] Weidmann P, De Myttenaere-Bursztein S, Maxwell MH, de Lima J. Effect on aging on plasma renin and aldosterone in normal man. Kidney International 1975; 8: 325 – 333
[87] Wu LT, Anthony JC. The estimated rate of depressed mood in US adults: recent evidence for a peak in later life. J.Affect.Disord. 2000; 60: 159 – 171
[88] Zhu Z, Tepel M, Spieker C, Zidek W. Effect of extracellular Mg^{2+}concentration on agonist-induced cytosolic free Ca^{2+} transients. Biochim.Biophys.Acta 1995; 1265: 89 – 92