Improvement of Sleep and Pituitary-Adrenal Inhibition After Subchronic Intranasal Vasopressin Treatment in Elderly Humans

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Abstract: Subchronic intranasal treatment with arginine-vasopressin (AVP) has been shown to exert a strong ameliorating effect on sleep and slow wave sleep (SWS) deficits in elderly. However, AVP is also a potent stimulus of the pituitary-adrenal stress system, which is usually inhibited during early, SWS-rich sleep. A disinhibition of pituitary-adrenal activity during sleep is correlated with aging and is considered a pathologic factor contributing to various age-related diseases. Here, we examined whether the beneficial effect of prolonged intranasal AVP administration on sleep in aged would be associated with a concomitant decrease in pituitary-adrenal inhibition and effects on other neuroendocrine features of sleep. Twenty-six healthy elderly (mean 72.9 yr) with mild sleep complaints were investigated in a placebo controlled double-blind study. One group was treated daily each morning and evening with intranasal AVP (2×20 IU) for 10 weeks, the other received placebo. During polysomnographical recordings taken at the beginning and end of the treatment period, blood was sampled every 15 min. Intranasal AVP increased SWS on average by +21.5 min ($p<0.02$). The effect persisted on the night after acute withdrawal of the peptide treatment with no rebound occurring. Notably, rather than increasing pituitary-adrenal activity, AVP decreased the early sleep cortisol nadir on average by 0.5 μg/dl ($p<0.05$). AVP did not induce any measurable changes in fluid balance or cardiovascular activity. Overall, results indicate a promoting effect of AVP on SWS in aged accompanied by a beneficial rather than impairing influence on the neuroendocrine pattern of sleep.

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A prominent feature of poor sleep in aged is its lack of slow-wave sleep (SWS) apart from frequent awakenings, and less pronounced a decrease in rapid-eye movement sleep.[1,2] A previous clinical study in aged revealed a dramatic improvement in sleep architecture after a three-month intranasal treatment with AVP.[3] Compared to a placebo treated group, elderly on AVP spent on average 4% more time in SWS, and also total sleep time increased on average by 41 min. The treatment was safe and side effects like hypertension or disturbances of fluid balance did not occur. However, sleep is also an endocrine event and endocrine activity was not measured in this study. Most important, pituitary-adrenal activity is inhibited during the early SWS-rich part of sleep so that cortisol plasma concentrations reach nadir values during this period.[4] This inhibition coincides with peak plasma concentrations of growth hormone (GH). In the second half of the night, when SWS is less likely, cortisol levels strongly increase to reach a maximum at the time of morning awakening.[5] Notably, the age-related impairment of sleep is associated with distinct alterations of this neuroendocrine pattern of sleep.[6–9] In particular, inhibition of pituitary-adrenal activity during early sleep weakens gradually with increasing age, and also GH release during sleep is distinctly decreased in aged. These alterations represent hallmarks of the age-related decrease in endocrine function and are of clinical relevance contributing distinctly to the morbidity in elderly people.[2,10] The age-related disinhibition of pituitary-adrenal activity during sleep has been considered to reflect deficient feedback within the hypothalamo-pituitary-adrenal (HPA) system as a long-term consequence of increased accumulation of life time stress, and also appears to be a factor contributing essentially to the development of age-related disorders such as metabolic syndrome and memory loss.[10–13]

AVP is known as an acute and potent stimulus of pituitary-adrenal activity. Naturally, AVP acts synergistically with corticotropin release hormone to regulate the response to various stressors.[14,15] On this background we wondered whether the improvement in sleep and SWS seen after prolonged intranasal treatment with AVP in the aged would be achieved at the expense of an increased pituitary-adrenal activity during sleep. For this purpose, the present study assessed effects of a subchronic intranasal

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AVP treatment in aged on sleep in conjunction with endocrine activity of the pituitary-adrenal axis and of the somatotropic axis. Moreover, possible unwanted side effects related to the pressor and antidiuretic activity of the peptide were monitored.

MATERIALS AND METHODS

Subjects

Subjects were recruited by advertisements in the local newspaper. Forty-two non-smokers participated in the study. The mean age was 72.9 yr (range 68–87 yr). They had no need of care, took no medication and had normal blood pressure. Cardiovascular, metabolic, neurologic and psychiatric diseases were ruled out by detailed history and physical examination, and after performing an electrocardiogram and routine laboratory tests including thyroid function tests. A score < 7 on Hamilton's Depression Scale[16] was used to exclude depression. At the sleep laboratory, sleep associated breathing and movement disorders were excluded before the experiment proper. Participants complained about mild insomnia as assessed by a standardized interview based on the Pittsburgh Sleep Quality Index (PSQI).[17] A score indicating mild insomnia and corresponding to PSQI values > 5 and < 10 was required to be eligible for the study. After screening, subjects were matched on the basis of age and sex and allocated to two treatment groups (AVP, Placebo). Of the 42 subjects included, 7 terminated the study prior to completion of the treatment phase for personal (two subjects) and medical reasons: in one subject gastric cancer was detected, one subject developed severe back pain, one subject of the placebo group developed hypertension, syncope occurred in two subjects (one of the placebo and the AVP group, respectively). Incomplete blood samples led to further exclusion of 9 subjects from parts of the analyses on sleep and endocrine parameters (due to temporary technical problems during at least two succeeding blood samplings through the wall, like kinking of the iv line, clotting of the venous access or accidental occlusion of the iv line by the subject himself). Including these subjects in the analysis of sleep yielded essentially the same results as for the restricted sample. However, for a reliable statement on the relation between sleep and endocrine activity, we decided to remove these subjects' data. Based on the strength of effects obtained in a similar foregoing study[3] the resulting group size of at least 11–12 was still considered sufficient to assess effects of the vasopressin treatment on the parameters of interest here. Mean age of participants was 73.0 yr (range 68–87 yr) in the placebo group (9 females, 10 males) and 72.8 yr (range 68–81 yr) in the AVP group (10 females, 6 males). Body mass indices were comparable in both groups (mean±SD: placebo vs. AVP: 26.17±1.00 vs. 25.93±0.80 kg/m^2). The local ethics committee approved the study. Each subject was paid and gave informed written consent prior to participation.

Procedure and design

An ad-libitum design as described in a foregoing study was applied.[3] Participants were asked to adhere to their individual sleeping habits like bed time, reading before lights off, time of getting up in the morning or daytime naps throughout the study. During experimental nights, subjects were allowed to sleep as long as desired. Whenever they decided to get up in the morning they gave a signal to the sleep laboratory personnel to turn on the lights.

During the baseline period of 2 days all subjects were treated with placebo (saline solution). After the second night (Night 2) subjects were randomly assigned to receive either AVP diluted in saline solution (arginine-vasopressin, Bachem, Heidelberg, Germany), or placebo (saline solution). Treatment allocation was kept double-blind. Treatments were taken daily for 10 weeks as two intranasal puffs in the morning after awakening and in the evening at least one hour before bedtime. In the AVP group each puff contained 10 IU AVP. Thus, subjects assigned to the AVP group received a daily dose of 40 IU AVP. Bottles with the test substance were stored in a refrigerator at 4° C and were replaced weekly. It was assured that with this storage method no significant degradation of the test substance occurs within 14 days. Treatment with the test substance was continued for 10 weeks. Subjects spent the last two nights of the treatment period (Nights 3,4) at the sleep laboratory (i.e., the spray was discontinued after it had been taken the last time on the evening before Night 4). In a subsample of 14 subjects, Night 4 was followed by another night (Night 5) to test effects of acute withdrawal of AVP on sleep (blood was not sampled on these additional nights).

For experimental nights, subjects arrived at the sleep laboratory three hours before the desired bedtime. After preparation for polysomnographical recordings and nocturnal blood sampling, subjects filled in a German version of a complaint list to assess possible physical and mental symptoms and side effects of the treatment.18 Blood pressure and heart rate were measured 30 min after arrival at the sleep laboratory, before lights were turned off and 10 min after awakening. After subjects went to bed at their individual bed times, sleep was recorded by standard polysomnography till subjects indicated that they wanted to get up. Night 1 and Night 3 served to accustom the subject to the laboratory setting. During Night 2 and 4, blood was collected via a forearm catheter connected to a thin plastic tube (volume 1.5 ml) enabling collection through the wall from an adjacent room without disturbing the subject's sleep. Blood was sampled every 15 min to determine GH

and cortisol and every 60 min for measurement of AVP, sodium, osmolality and a blood count. Total amount of blood sampled on a night was about 300 ml. To prevent clotting between blood taking, the collecting tube was slowly flushed with 300 ml of saline solution during the night. 30 min after awakening, subjects performed a letter-cancellation test to assess attention.[19] Thereafter, mood and feelings of activation were examined by a multidimensional adjective checklist.[20] Fluid balance was assessed in a subsample of 19 subjects. These subjects were asked to collect their urine for a 24-h interval before treatment and at the end of the treatment period. Collecting periods started from awakenings after Night 1 and Night 3, respectively. Specific weight and urine osmolality were measured in aliquots of daytime and nighttime urine, respectively. In the morning after Night 2 and 4, body weight was measured, and potassium, calcium, creatinine, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, cholesterol, triglyceride, differential blood count, insulin-like-growth factor (IGF)-1 were determined in additional blood samples.

In a diary subjects reported daily subjective sleep quality, duration of sleep, frequency of nocturia, and daytime naps. Subjective sleep quality of the foregoing night was assessed via self-rating on an 11-point rating scale ranging from –5 (very poor sleep) to +5 (very good sleep). At weekly intervals subjects were asked for side effects, and blood pressure and heart rate were measured.

Recordings

For standard polysomnographical recordings (Nicolet EEG 1A97, Nicolet, Madison, WI, USA) Ag/AgCl EEG electrodes were placed at C3 and C4 referenced to an electrode at the nose. Blood pressure and heart rate were measured by an automatic manometrical device (Boso "Medicus", Bosch und Sohn, Jungingen, Germany). For determining hormone levels the following commercially available tests were used: Vasopressin: radioimmunoassay after extraction (Bühlmann Laboratories, Allschwill, Switzerland, sensitivity 0.35 pg/mL (0.32 pmol/L), intraassay coefficient of variation (CV) $< 11.2\%$ between 0.35 and 20.0 pg/mL (0.32 and 18.4 pmol/L, respectively)). Cortisol: radioimmunoassay (DPC Biermann, Bad Nauheim, Germany, sensitivity 0.2 μg/dL (5.5 nmol/L), intraassay CV $< 5.1\%$ between 1 and 50 μg/dL (27.6 and 1379.5 nmol/L, respectively)). GH: radioimmunoassay (DPC Biermann, Bad Nauheim, Germany, sensitivity 0.9 ng/mL (1.8 mLU/L), intraassay CV $< 5.9\%$ between 1.1 and 10.9 ng/mL (2.2 and 21.8 mLU/L, respectively)). IGF-1: radioimmunoassay after acid-ethanol extraction of binding proteins (Nichols, Geneva, Switzerland, sensitivity 13.5 ng/mL (1.8 nmol/L), intraassay CV 3%)). Interassay coefficient of variation of all assays was below 10% and all samples from an individual were measured in duplicate in the same assay. Blood count, serum sodium and osmolality were measured by routine laboratory methods.

Data reduction and analysis

Sleep recordings were evaluated according to Rechtschaffen and Kales[21] by two experts, who were not aware of the treatment conditions. The following sleep parameters were determined: Sleep onset latency (SOL, first occurrence of sleep stage 1 followed by sleep stage 2), sleep period time (SPT, time from sleep onset until final awakening defined as last occurrence of any sleep stage with the exception of "Wake"), total sleep time (TST, time spent in sleep stages 1,2,3,4 (S 1,2,3,4) and REM sleep between sleep onset and final awakening), sleep efficiency (SPT minus time awake relative to total SPT (%)), time (of SPT) spent awake after sleep onset (WASO), in S 1, 2, 3, 4, in SWS (S3+S4) and REM sleep (in min and %), and latency of the first occurrence of each sleep stage (with reference to sleep onset). In addition, time spent in the different sleep stages (in min and %), was determined separately for the first and the second half of SPT. Moreover, for each sleep stage the number of shifts into this stage and the average duration of an epoch in that stage were determined. For cortisol and GH the following parameters were calculated: average cortisol and GH levels during the whole night and separately for the first and second half of SPT; maximum cortisol and GH concentration and minimum (nadir) cortisol concentration (highest and lowest absolute value, respectively); latency of the maxima of cortisol and GH and of the cortisol nadir, calculated with reference to sleep onset.

Statistical analysis

Statistical analysis, in general, relied on analyses of covariance (ANCOVA) including a group factor "Treatment" (AVP versus Placebo). The BMDP statistical software (2V) was used for ANCOVA, which also included tests to assure normal distribution in the data set. Data obtained at Night 2 served as covariate and the data obtained at Nights 4 and Night 5, respectively, were the dependent variables (Nights 1 and 3 were adaptation nights and were not included in the analysis). ANCOVA on variables recorded weekly (blood pressure, heart rate) or daily (sleep duration, bed time, waking time) included an additional factor "Time". Effects of vasopressin on frequency of nocturia, daytime naps, and subjective sleep quality were evaluated by the Kruskal-Wallis test. Means±SD reported are baseline adjusted as derived from ANCOVA. A p-value < 0.05 was considered significant. Depending on the type of hypothesis, two-sided and (when indicated) one-sided tests were employed. Degrees of freedom were corrected following the Greenhouse-Geisser procedure.

RESULTS

Sleep

At baseline, both treatment groups were comparable with regard to SOL, SPT, sleep efficiency and time spent in each sleep stage. Subjects displayed the typical age related sleep pattern during baseline Night 2, and on Night 4 after placebo treatment (Table 1) as indicated by a prolonged time awake and in stage 1 sleep and relatively short time in SWS and in REM sleep. In Night 4 after 10 weeks of AVP treatment, time spent in SWS was significantly increased on average by 21.5 min, as compared to placebo ($p<0.02$ for one-sided paired comparison, Table 1 and 2, Fig. 1). SPT and REM sleep were also prolonged on average by 18.9 min and 11.6 min, respectively. However, due to a high variability, these effects did not reach significance. The increase in SWS following AVP was associated with reduced time spent in sleep stage 2 mainly in the first half of the night ($p<0.02$).

On Night 5, when treatments had been discontinued and no blood was sampled during sleep in a subsample of 14 subjects, SPT and time in SWS was generally longer, possibly due to less disturbance by the sampling procedure (Table 1). The increase in SWS in the AVP group was not only well preserved, but was nearly doubled during this night (Table 1, Fig. 1). Also, SPT was increased on Night 5 on average by 75 min in the AVP group as compared to the placebo group ($p<0.04$). Additionally, Night 5 indi-

TABLE 1. Sleep parameters, whole night

	Night 4 (last night on treatment)					Night 5 (first night post-treatment)				
	Placebo (n = 14)		AVP (n = 12)			Placebo (n = 6)		AVP (n = 8)		
	Mean	SD	Mean	SD	p value	Mean	SD	Mean	SD	p value
Sleep onset latency (min)	**25.1**	23.2	**37.5**	23.2		**20.2**	12.0	**17.5**	11.9	
Δ-value	+9.5	14.1	+21.8	31.2		+2.3	20.7	+1.4	5.5	
Sleep period time (min)	**397.3**	71.5	**416.2**	71.4		**437.8**	66.9	**512.9**	67.6	**<0.04**
Δ-value	−15.8	83.1	+21.6	65.2		+42.8	81.3	+99.4	64.2	
Total sleep time (min)	**320.7**	74.8	**330.2**	68.9		**370.9**	54.4	**450.5**	70.5	**<0.04**
Δ-value	−10.2	70.1	+22.8	73.5		+37.5	75.7	+101.3	68.1	
Sleep efficacy (%)	**80.6**	9.4	**79.6**	10.0		**84.7**	7.4	**87.5**	10.8	
Δ-value	−2.7	9.0	−4.6	9.9		−6.4	8.6	+3.6	10.8	
REM latency (min)	**44.8**	50.1	**45.2**	49.8		**40.6**	51.9	**101.4**	51.2	**<0.05**
Δ-value	+2.9	55.0	−8.3	69.0		−9.6	11.6	+11.5	40.3	
Time of sleep stages (min)										
WASO	**76.6**	51.6	**86.0**	51.9		**66.9**	47.0	**62.4**	46.5	
Δ-value	+5.2	46.5	+23.0	77.0		+14.5	83.0	−11.5	43.4	
S1	**46.6**	36.7	**42.5**	37.1		**60.5**	32.5	**47.7**	27.4	
Δ-value	+3.8	16.6	−3.2	26.3		+7.3	50.0	−4.4	22.6	
S2	**204.0**	46.0	**184.5**	46.1		**213.1**	32.0	**252.3**	40.2	**<0.07**
Δ-value	−11.5	58.0	−24.0	62.1		+22.6	42.4	+48.8	41.5	
S3	**21.7**	19.5	**35.4**	19.9	**<0.05**	**18.0**	36.8	**56.1**	37.8	**<0.05**
Δ-value	−4.5	19.6	+15.3	22.9		−0.8	35.4	+36.0	33.6	
S4	**0.2**	10.8	**14.2**	11.1		**5.3**	7.5	**10.4**	7.1	
Δ-value	−3.2	8.0	+5.8	14.4		−2.3	5.5	+4.0	8.1	
SWS (S3 + S4)	**21.3**	22.1	**42.8**	22.1	**<0.02**	**22.3**	39.5	**69.3**	33.5	**<0.02**
Δ-value	−7.7	13.5	+21.2	18.3		−3.1	20.4	+40.0	20.8	
REM	**48.8**	20.6	**60.4**	19.0		**75.0**	35.5	**81.2**	37.6	
Δ-value	−5.5	20.5	+8.0	25.1		−3.5	57.6	+26.6	15.5	
M	**20.2**	8.2	**16.1**	8.3		**18.9**	5.4	**17.6**	10.5	
Δ-value	−0.5	12.5	−4.4	9.5		+0.5	9.2	−2.3	6.6	

Left (Night 4): sleep in 26 healthy elderly subjects after 10 weeks of intranasal treatment with AVP and placebo. Sleep was assessed on the night after the last intranasal treatment in the evening (Night 4). Right (Night 5): In a subsample of 14 subjects withdrawal effects were investigated in the night following the last treatment night. All values are adjusted with reference to a baseline night before the 10-week treatment interval, as derived from ANCOVA. In addition differences from baseline Night 2 (Δ-values) are provided for each parameter, indicating the direction of effects with respect to baseline. Significance for the difference ($p<0.05$) between the effects of the treatments and statistical trends ($p<0.1$) are indicated as determined by ANCOVA. Sleep onset latency: time between lights off and the onset of the first epoch of sleep stage 1 followed by sleep stage 2; sleep period time (SPT): time between sleep onset and final awakening; total sleep time (TST): time spent asleep between sleep onset and final awakening; sleep efficacy defined by SPT minus time awake (WASO) relative to SPT (%); WASO: time awake after sleep onset; S1, S2, S3, S4: sleep stage 1, 2, 3 and 4; SWS: slow wave

TABLE 2. Sleep stages, separately for the first and second half of the night

	Night 4 (last night on treatment)					Night 5 (first night post-treatment)				
	Placebo (n = 14)		AVP (n = 12)			Placebo (n = 6)		AVP (n = 8)		
	Mean	SD	Mean	SD	p value	Mean	SD	Mean	SD	p value
Sleep stages 1st half (%)										
WASO	**15.4**	15.7	**19.9**	16.0		**14.2**	12.3	**11.4**	10.4	
S1	**10.8**	4.5	**8.8**	4.6		**13.3**	10.8	**8.9**	2.9	
S2	**57.0**	11.2	**47.9**	11.5	**<0.02**	**53.3**	15.9	**45.2**	10.6	
S3	**7.7**	7.9	**11.3**	8.0		**4.2**	11.8	**14.1**	11.3	
S4	**0.0**	3.7	**2.6**	3.4	**<0.03**	**1.7**	5.9	**4.2**	2.8	
SWS (S3 + S4)	**7.7**	8.9	**13.8**	9.0	**<0.05**	**5.9**	14.7	**18.3**	9.3	**<0.05**
REM	**9.1**	5.2	**9.7**	5.1		**13.3**	6.9	**16.2**	6.8	
M	**5.0**	2.2	**2.8**	2.0		**3.8**	1.2	**4.2**	2.6	
Sleep stages 2nd half (%)										
WASO	**20.6**	15.2	**22.0**	15.7		**16.1**	11.2	**14.5**	11.0	
S1	**12.3**	5.6	**11.6**	5.5		**14.7**	3.9	**11.5**	4.0	
S2	**46.3**	12.7	**40.8**	12.9		**45.1**	7.8	**52.2**	7.9	**<0.06**
S3	**5.0**	7.4	**5.3**	8.0		**2.6**	5.2	**4.5**	5.1	
S4	**0.1**	0.4	**1.3**	0.3		**0.6**	2.5	**0.8**	0.6	
SWS (S3+S4)	**5.1**	8.2	**6.6**	8.3		**3.2**	3.4	**5.3**	2.8	
REM	**15.7**	7.4	**19.0**	8.1		**20.9**	10.5	**16.5**	10.6	
M	**5.7**	0.4	**4.6**	0.3		**4.2**	0.7	**2.8**	1.7	

Left (Night 4): sleep in 26 healthy elderly subjects after 10 weeks of intranasal treatment with AVP and placebo. Sleep was assessed on the night after the last intranasal treatment in the evening (Night 4). Right (Night 5): In a subsample of 14 subjects withdrawal effects were investigated in the night following the last treatment night. All values are adjusted with reference to a baseline night before the 10-week treatment interval, as derived from ANCOVA. Significance for the difference ($p<0.05$) between the effects of the treatments and statistical trends ($p<0.1$) are indicated as determined by ANCOVA. For abbreviations refer to Table 1.

cated a significantly prolonged REM latency in the AVP group ($p<0.05$).

Cortisol, GH and IGF-1

Cortisol concentrations were, as expected, distinctly lower during early than late sleep. There were no hints at an elevation of nocturnal cortisol concentrations following AVP. On the contrary, although our group of aged showed only slightly elevated cortisol nadir concentrations as compared to the normal range observed in young subjects, AVP further enhanced suppression of cortisol release as indicated by 0.5 μg/dl (23.8 %) reduction in the cortisol nadir ($p<0.04$, Table 3, Fig. 1). On the placebo condition as well as at baseline, secretion of GH in the elderly was as expected lower than typically observed in young healthy subjects (Table 3). After intranasal AVP average GH levels during SPT increased by 0.8 ng/ml, which approached significance ($p<0.08$). The same tendency towards increased GH release following AVP treatment was obtained in separate analyses of the late part of the night (Table 3). Concentrations of IGF-1 measured in the morning after experimental nights did not reflect an effect of AVP treatment (mean±SD placebo vs. AVP: 84.8±16.5 vs. 88.3±17.0 pg/mL, n.s.).

Indicators of fluid balance and laboratory findings

Plasma concentrations of AVP in night 4 after AVP treatment were significantly elevated at the time when lights were turned off, due to prior application of the peptide (mean±SD placebo vs. AVP: 1.55±2.28 vs. 4.16±2.60 pg/mL, Figure 2). Later, AVP concentrations decreased gradually and approached the levels of the placebo condition in the morning hours. Average AVP concentrations for the total SPT tended to be higher in the AVP than placebo group (2.84±1.70 vs. 1.71±1.65 vs. pg/mL, $p<0.1$). Measurement of sodium, osmolality and hematocrit did not reveal any treatment effects, and values were entirely within the normal range (Table 4). Sodium increased towards the end of the night, while hematocrit and osmolality slightly decreased. 16 subjects had to be excluded from evaluation of urine parameters due to incorrect sampling. In the remaining subjects urine output was comparable on both treatment conditions. During daytime subjects voided more urine than during night time, but no treatment effects were detected. Also, osmolality and specific weight in the urine revealed no effects of intranasal AVP (Table 4). Body weight was not significantly changed by AVP treatment. Furthermore, AVP treatment did not exert any effect on plasma

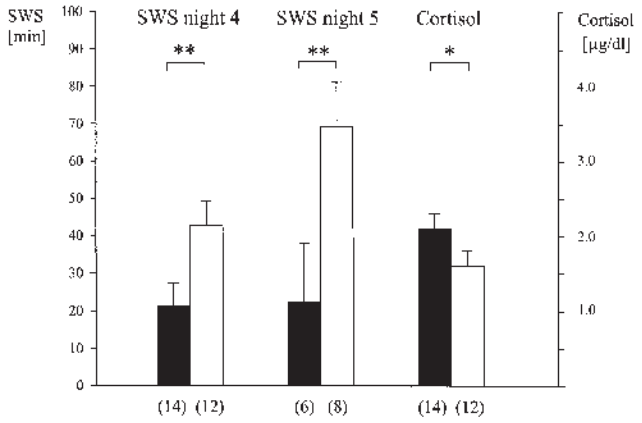

FIG. 1. Effects of AVP on SWS and cortisol nadir values. Mean (±SEM) time spent in SWS and associated cortisol nadir values after a 10-week treatment with intranasal AVP (empty bars) and placebo (black bars) in 26 elderly subjects. Effects on SWS are indicated separately for Night 4 (last night of treatment) and Night 5 (first night after withdrawal of treatment). Sample size is indicated below the bars. * $p<0.05$, ** $p<0.02$ for comparison with placebo condition as derived from ANCOVA.

concentrations of potassium, calcium, creatinine, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, cholesterol, triglyceride and differential blood counts determined in the morning after experimental nights.

Cardiovascular measures

Systolic blood pressure was slightly elevated following AVP administration in recordings obtained shortly before lights were turned off (Table 5), due to the acute application of the test substance one hour before. In the morning as well as in measurements before substance administration, differences between AVP and placebo treated subjects remained non-significant. Also, diastolic blood pressure and heart rate at no time indicated any treatment effect. Examination of blood pressure and heart rate once a week during the 10-week treatment period revealed values in the normal range and no treatment related differences.

Self-ratings, attention and sleep diary

A self-rating scale of complaints indicated only the general presence of mild physical and mental disturbances in the subject sample, and did not reveal any side effects of the AVP treatment. Also mood assessed by an adjective check list, and attention assessed by a letter cancellation test in the morning after experimental nights did not indicate any effects of AVP treatment. Table 6 summarizes results of the diary kept by the subjects during the 10-week treatment period. Reported sleep time at home was collapsed across the whole treatment period as well as across the first two weeks and the last two weeks. Also, bed time and rising time were assessed. Altogether these parameters were not influenced by AVP treatment. Rated sleep quality, and frequency and estimated duration of naps did not change. The frequency of nocturia on average was low (AVP: 1.04±0.92; placebo: 0.83±0.57) and there was no significant reduction following AVP.

TABLE 3. Endocrine parameters

	Placebo (n = 14)		AVP (n = 12)		
	Mean	**SD**	**Mean**	**SD**	**p value**
GH (ng/ml)					
Peak	**2.2**	3.0	**3.7**	2.8	
Δ-value	**−0.5**	1.7	**+0.9**	3.5	
Peak latency (min)	**94.2**	105.8	**97.0**	98.3	
Δ-value	**−13.2**	133.2	**−63.9**	175.7	
Average levels during:					
SPT	**1.0**	0.7	**1.8**	0.7	**<0.08**
Δ-value	**−0.1**	0.2	**+0.3**	0.9	
1st half	**1.1**	1.1	**1.6**	1.0	
Δ-value	**−0.2**	0.3	**+0.1**	0.2	
2nd half	**0.9**	0.4	**1.2**	0.3	**<0.08**
Δ-value	**0**	0.1	**0**	0.3	
Cortisol (µg/dl)					
Peak	**16.2**	3.8	**16.1**	3.6	
Δ-value	**−2.6**	5.3	**−2.1**	5.7	
Peak latency (min)	**290.9**	105.7	**329.1**	100.3	
Δ-value	**−28.3**	164.1	**+0.6**	79.3	
Nadir	**2.1**	0.7	**1.6**	0.7	**<0.04**
Δ-value	**+0.1**	0.7	**−0.5**	**0.8**	
Nadir latency (min)	**113.3**	47.6	**99.7**	46.7	
Δ-value	**+33.1**	91.9	**+2.6**	14.7	
Average levels during:					
SPT	**6.8**	1.5	**6.8**	1.4	
Δ-value	**−1.1**	1.7	**−0.8**	2.1	
1st half	**4.1**	1.5	**3.7**	1.7	
Δ-value	**−0.8**	3.2	**−0.1**	1.2	
2nd half	**8.9**	2.6	**10.0**	2.4	
Δ-value	**−0.6**	3.0	**−1.7**	4.1	

Baseline adjusted means (±SD) of endocrine parameters during nocturnal sleep in elderly subjects following 10 weeks of intranasal administration of AVP and Placebo as derived from ANCOVA. Peak concentrations of GH and cortisol and peak latencies with reference to lights off as well as nadir concentrations of cortisol and its latency are indicated. Average concentration of GH and cortisol were determined for sleep period time as well as separately for the first and second half of sleep time. Also, the difference with reference to the baseline night (Δ-values) is indicated for each parameter. P values are shown for private comparisons as derived from ANCOVA.

DISCUSSION

The present study examined effects of daily intranasal AVP administration for 10 weeks on sleep and sleep-associated endocrine activity in healthy elderly humans. During baseline nights, subjects displayed characteristic age-related changes in sleep architecture consistent with previous

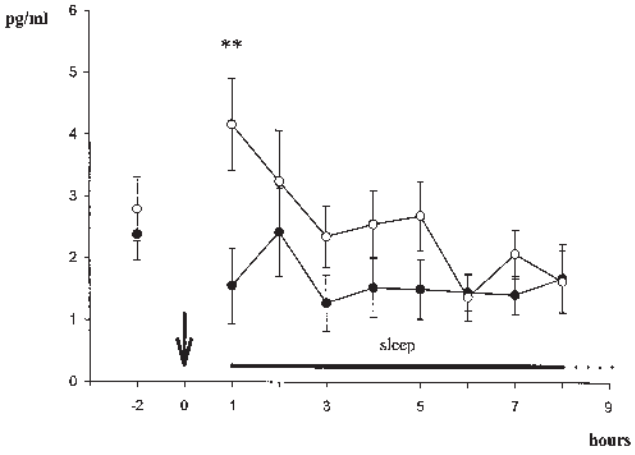

FIG. 2. Plasma AVP levels at night. Plasma concentrations of AVP (mean±SEM) measured hourly during an experimental night after 10 weeks of intranasal treatment with a daily dose of 40 IU AVP (20 IU in the morning, 20 IU in the evening; open circles, n=14) and placebo (closed circles, n=12). Treatments were administered 1 hour before lights were turned off (black arrow at '0 h'). Also, values are indicated for a measurement 2 hours prior to the acute evening administration of the substance (-2 h). Black bar indicates sleep period time. ** $p<0.01$ for pairwise comparison between the effects of the treatment.

studies in aged humans.[2,6–10,22] Ten weeks of intranasal treatment with AVP substantially enhanced time in SWS, on average by 21.5 min. On the night after withdrawal of treatment no rebound occurred, but time in SWS was even further increased if compared with the placebo condition. A most important finding was that AVP treatment did not induce any signs of pituitary-adrenal activation during sleep but, on the contrary, significantly enhanced inhibition of pituitary-adrenal activity during early SWS-rich sleep, as indicated by significantly reduced plasma cortisol concentrations in this period. Moreover, the increase in SWS following treatment with AVP was associated with a tendency towards enhanced release of GH during sleep. There was no evidence for any adverse effects of prolonged intranasal AVP treatment on parameters of cardiovascular activity and body fluid balance.

Sleep changes in the present study concentrated on SWS, which increased on average by 21.5 min, and was paralleled by a reduction in time spent in sleep stage 2 during the first half of the night. This result confirmed foregoing data of an increase in SWS averaging 22 min after 3 months of intranasal AVP.3 In that study, TST and REM sleep also were significantly extended by 41 and 10 min respectively, following AVP. Here, TST and time in REM sleep increased on average by 9.5 min and 11.6 min, respectively, after AVP. However, these changes were not statistically significant because of a higher variability in the data. This failure was probably caused by the ongoing blood sampling during sleep which was not employed in the previous study and represents an additional strain on the elderly subjects even after careful adjustment to the procedure. This view is further supported by the results of Night 5 which took place after treatments had been discontinued and which did not include blood sampling. In general, both subjects of the AVP and the placebo group slept better on this night. However, the effect of AVP on SWS did not only prevail, but increased so that subjects of

TABLE 4. Indicators of fluid balance

	Placebo		AVP	
	Mean	**SD**	**Mean**	**SD**
	(n = 9)		**(n = 10)**	
Urine output				
Day time volume [ml]	**733**	459	**875**	310
Nigh time volume [ml]	**479**	159	**627**	291
Osmolality [mosmol/kg]	**700**	279	**596**	189
Specific weight	**1024**	9.3	**1023**	7.7
Plasma parameters	**(n = 19)**		**(n = 16)**	
Sodium [mmol/L]	**141.34**	1.7	**141.18**	1.7
Osmolality [mosmol/kg]	**293.8**	4.4	**292.9**	4.0
Hematocrit	**0.373**	0.02	**0.365**	0.02

Urine volume, osmolality and specific weight after 10 weeks of intranasal treatment with AVP and placebo. Urine was collected from the morning after the adaptation night till lights off on the experimental night (day time urine), and from then on till the morning after the experimental night (night time urine). Means (±SD) as derived from ANCOVA are presented. Differences between treatment groups were not significant.

TABLE 5. Cardiovascular parameters

	Placebo (n = 19)		AVP (n = 16)		
	Mean	**SD**	**Mean**	**SD**	**p value**
Systolic blood pressure [mmHg]					
Before admin.	**136.8**	15.5	**140.2**	15.6	
1 h after admin.	**126.3**	16.6	**138.6**	16.0	<0.05
At awakening	**134.1**	12.2	**138.5**	12.4	
Diastolic blood pressure [mmHg]					
Before admin.	**82.1**	10.5	**82.9**	10.4	
1 h after admin.	**76.2**	9.2	**75.8**	8.8	
At awakening	**78.3**	10.0	**80.5**	10.0	
Heart rate [beats/min]					
Before admin.	**72.3**	10.0	**73.3**	10.0	
1 h after admin.	**66.2**	7.0	**66.1**	6.8	
At awakening	**68.2**	9.2	**70.0**	9.2	

Baseline adjusted mean (±SD) blood pressure and heart rate after 10 weeks of intranasal AVP and placebo, as derived from ANCOVA. Values for pairwise comparisons between effects of AVP and placebo are presented.

TABLE 6. Diary reports

	Placebo (n = 19)		AVP (n = 16)	
	Mean	**SD**	**Mean**	**SD**
Sleep time [h:min]				
Total period	**7:41**	0:48	**7:37**	0:48
a) Day 1–14	**7:47**	0:50	**7:46**	0:44
b) Day 56–70	**7:39**	0:48	**7:37**	0:44
Nocturia [frequency/night]				
Total period	**0.83**	0.57	**1.04**	0.92
a) Day 1–14	**1.05**	0.83	**1.07**	0.96
b) Day 56–70	**0.65**	0.44	**1.07**	1.00
Sleep quality [rating]				
Total period	**+2.15**	2.41	**+2.00**	2.00
a) Day 1–14	**+1.81**	2.44	**+2.07**	2.22
b) Day 56–70	**+2.46**	2.63	**+2.13**	2.00
Day time naps [min]				
# of subjects taking naps	**6**		**6**	
Total period	**30.0**	20.1	**20.0**	11.3
a) Day 1–14	**29.8**	20.0	**20.3**	11.4
b) Day 56–70	**32.4**	20.8	**19.4**	17.6

Mean (±SD) of reported sleep time, nocturia, sleep quality and occurrence of daytime naps in subjects treated for 10 weeks with intranasal AVP and placebo. Daytime naps were analyzed only for subjects reporting more than two naps per week. Average values for the total 10-weeks treatment period as well as for the first and last 14 days of the treatment period are shown. Pairwise comparisons of differences between treatment groups were not significant.

the AVP group on this night spent on average 47.0 min more time in SWS than the placebo subjects. Moreover, the AVP treated group showed a significantly enhanced REM latency and prolonged TST (on average by 79 min) on this night, as compared with the placebo group. Results of Night 5 are also interesting, because sleep-promoting effects of AVP persisted (and were even enhanced) although treatment was discontinued 24 h before. The mechanisms underlying this effect are presently obscure. A possible explanation is that during Night 5 the sleep disrupting effect that has been found to be associated with acute administration of AVP23 is missing while the effect of chronic administration (e.g., changes in receptor expression) are still present. The lack of rebound insomnia deserves further notice, because substances frequently given for treatment of insomnia in elderly persons, like benzodiazepines, show strong rebound effects after termination of the treatment.[24,25]

The significant decrease in cortisol nadir concentrations during the early part of sleep following AVP treatment appears even more remarkable in light of the fact that our sample of aged persons during the baseline condition did not reach the high levels observed in comparable groups of elderly in foregoing studies.[7,9] Although slightly higher than those observed in young subjects, in comparison with previous data,[9] the cortisol nadir values in the present group of aged appeared to be representative for persons between 51–60 yr of age rather than for the age between 68–87 yr as they actually were. Thus, cortisol nadirs characterized our subjects as a rather youthful sample. On this background, the reduction in cortisol nadir values indicates an enhancing effect of prolonged intranasal treatment with AVP on the inhibition of the pituitary-adrenal activity known to be established during early sleep and particularly during periods of SWS.[4,26,27] This inhibition is most likely mediated via a supraordinate limbic control over the HPA-system and becomes weakened in the course of aging.[7–9] Yet, a comprehensive characterisation of HPA activity in our elderly subjects clearly would have required also examination of secretory responses to CRH challenge, in the presence and absence of dexamethasone suppression.[28]

Two factors may be relevant for integrating the surprising outcome that rather than stimulating the HPA-system, AVP induced signs of improved inhibition of this system during sleep: the duration of the treatment and the intranasal application of the substance. Previous studies have pointed to opposite effects of AVP depending on whether acute effects or effects after prolonged treatment were examined. Thus, acute administration of AVP was shown to worsen sleep and increase signs of arousal during sleep,[23] whereas subchronic administration revealed improvement in sleep architecture. Accordingly, the inhibiting effect observed here for the first time after AVP treatment is probably related to the prolonged duration of the treatment. Moreover, the use of an intranasal route of administration probably preferentially strengthened influences of the peptide on central nervous mechanisms. A direct action on brain structures is supported by experiments indicating a large and significant accumulation of AVP in the cerebrospinal fluid in humans after intranasal administration.[29–31]

A mechanism which could explain how AVP increases both SWS and pituitary-adrenal inhibition during early sleep refers to its action on mineralocorticoid receptors (MR). In rats, AVP has been shown to stimulate the expression of corticosteroid receptors in limbic and hippocampal brain regions.32 Moreover, blocking of hippocampal MR in humans (by canrenoate) reduced SWS and increased cortisol nadir values during early sleep,[33–36] while MR stimulation increased SWS.[37] In light of these findings, it is tempting to suggest that the subchronic intranasal treatment with AVP improves SWS and strengthens associated inhibitory control over pituitary-adrenal activity by stimulating expression of limbic-hippocampal MR. Alternatively, the AVP treatment may compensate for an age related loss of vasopressinergic neurons that has been demonstrated to affect selectively the suprachiasmatic nucleus of the hypothalamus.[38] This nucleus plays a key role in the regulation of the sleep-wake cycle.[39] Enhanced AVP levels in this region could improve sleep by

enhancing the amplitude of the sleep-wake rhythm. Similar mechanisms may account for the signs of increased GH release during sleep after AVP, although overall the improving effect of AVP on GH release showed large variability and needs further confirmation.

The present study also aimed at monitoring possible side effects of intranasal AVP treatment on cardiovascular function and body fluid homeostasis. Systolic blood pressure increased acutely within 1 hour after administration of AVP, measured shortly before lights were turned off. However, the increase was small and remained within the normal range. Also, there were no parallel changes in diastolic blood pressure and heart rate. Furthermore, data collected during weekly visits of the subjects at the sleep laboratory (usually around noon) did not provide any hints at a tonic elevation of blood pressure during AVP treatment, but showed values completely within the normal range. None of the measures taken to assess fluid balance, including serum sodium concentration, osmolality, hematocrit, urine output and body weight provided any evidence for a possible change in fluid balance during AVP treatment. There were neither hints at possible acute effects nor at more enduring effects of the treatment. Thus long lasting changes in fluid balance and an increase of blood pressure following AVP treatment were not observed, although such adverse effects cannot be entirely ruled out considering the limited size of our sample and the limited duration of exposure to the treatment.

Together, the present results are promising in that they demonstrate improved sleep in conjunction with signs of improved inhibitory control over pituitary-adrenal activity during sleep. Considering cortisol nadir values as reflexion of the adverse effects of the cumulative impact of stressful life events on mental and physical health and considering also the short period of treatment in this study compared to the long time during which the disturbances of sleep and endocrine activity developed, the changes observed here suggest that a significant beneficial effect on the neuroendocrine architecture of sleep can be achieved by AVP. However, overall the significance of the improving influence of sleep still needs to be further substantiated also in the light of the fact that here subjective ratings of sleep quality in the diary reports did not show a difference to placebo.

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