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Neuropeptides: Effects on Paradoxical Sleep and Theta Rhythm in Rats

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URBAN, I. AND D. DE WIED. *Neuropeptides: effects on paradoxical sleep and theta rhythm in rats.* PHARMAC. BIOCHEM. BEHAV. 8(1) 51–59, 1978. – In a search for the mechanism by which neuropeptides influence the maintenance of avoidance behavior, a study was done of the effects of ACTH 4–10, arginine^{8}-vasopressin (AVP), desglycinamide^{9}-arginine^{8}-vasopressin (DG–AVP), and of AVP antiserum on paradoxical sleep (PS) production and on theta activity generated during PS. Brattleboro rats which were homozygous (HO–DI) and heterozygous (HE–DI) for diabetes insipidus and homozygous normal (HO–NO) animals were used. The absence of AVP in HO–DI animals did not interfere with the production of PS during 8 hr recording sessions. However, it was found that the hippocampal theta activity of these animals exhibited significantly lower mean and peak frequencies than that of HE–DI or HO–NO rats. The deviation in the frequency content of the theta activity of HO–DI animals could be temporarily normalized by intraventricular injection of DG–AVP or ACTH 4–10. Inactivation of AVP in the brain of HO–NO rats by intraventricularly administered AVP antiserum induced hippocampal rhythmicity of the same frequency composition as that of HO–DI animals. Furthermore, intracerebroventricular administration of DG–AVP or ACTH 4–10 which both facilitate resistance to extinction of avoidance behavior [8,14], increased the proportion of high theta frequencies, whereas treatment with [D-phe^{7}] ACTH 4–10, which facilitates extinction of active avoidance behavior [8], decreased the proportion of high frequency components in hippocampal activity of HE–DI animals. In addition, [D-phe^{7}] ACTH 4–10 decreased the amount of high and increased the amount of low frequencies in the theta rhythm of HO–NO rats. None of the peptides tested appreciably affected the amount of PS. The experiments suggest that the increased resistance to extinction of avoidance behavior seen after the administration of neuropeptides related to vasopressin and ACTH could result from a longlasting increase in excitability induced by these substances in midbrain limbic circuits.

Paradoxical sleep Hippocampal theta activity Neuropeptides ACTH 4–10 AVP DG–AVP AVP antiserum

SOME EXPERIMENTS done in recent years have implicated neuropeptides of hypothalamo-hypophyseal origin in various brain functions. That the brain itself is a target for these principles was apparent from the behavioral disturbances seen in rats whose adenohypophysis or whole hypophysis had been removed [9, 12], in animals with hereditary diabetes insipidus (DI) and incapable of synthetizing vasopressin [13] or in rats in which the action of vasopressin in the brain was neutralized by administration of specific vasopressin antiserum [56]. The impairment in acquisition and maintenance of conditioned behaviors which is associated with the absence of pituitary principles can easily be corrected by treatment with ACTH, MSH, vasopressin and fragments of these polypeptides; the fragments themselves are devoid of the classical endocrine and metabolic effects of the parent hormones [10,11].

Hippocampal theta rhythm seems to reflect a functional state of the brain which facilitates learning and memory formation. Thus, the amount of theta activity of hippocampal origin significantly increased in some cortical and subcortical structures during learning in an appetitive approach situation [16]. Landfield *et al.* [27] found a positive correlation between the amount of cortical theta rhythm generated by rats in the first half hour after the training in a one trial passive avoidance task and the rate of retention two days after the training. Furthermore, post-trial injections with a number of centrally acting drugs, e.g., amphetamine, nicotine, eserine, pentamethylenetetrazol, strychnine, etc., resulted in superior retention of conditioned behaviors [33,37]. Most of these compounds had been shown to enhance the cortical and hippocampal theta rhythm [29, 32, 33].

Other studies pointed out that paradoxical sleep (PS) was of great importance for memory consolidation processes. Deprivation of PS in the period following training led to disturbances in the preservation of acquired responses [17, 19, 30, 31, 43] and exposure of rats to various learning situations was associated with increased PS production [20, 21, 34].

Brattleboro rats with hereditary hypothalamic diabetes insipidus offer a suitable model to study the mechanism underlying memory processes. A genetic defect in vasopressin synthesis in these animals [54] has been shown to be associated with memory dysfunction [13]. To explore the possibility that neuropeptides may affect the maintenance of avoidance behavior by modulating the activity of the theta generating substrate and/or by altering the production of PS, we have examined the effect of various

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neuropeptides on theta activity and amount of PS in Brattleboro rats. The spontaneous occurrence of theta rhythm during PS created a situation in which both processes could be studied in parallel in the same animal. The frequency composition of theta activity was selected as a measure of functional changes of the neural network participating in the theta rhythm generation in the presence or absence of neuropeptides while the amount, number and duration of PS episodes served to assess the influence of neuropeptides on PS production.

METHOD

Animals

Male rats of the Brattleboro strain, weighing from 200 to 250 g, were supplied by Central Breeding Laboratories TNO, Zeist, The Netherlands. The twenty-four hour water intake was measured before the actual experiment. The water intake was: for HO–DI rats (n = 10), 69.0 ± 5.1 ml/24 hr/100 g; for HE–DI rats (n = 10), 18.4 ± 0.8 ml/24 hr/100 g; for HO–NO rats (n = 7), 17.4 ± 1.9 ml/24 hr/100 g.

Experimental Procedure

Rats were anesthetized with sodium pentobarbital (Nembutal^{R}) in a dose of 6 mg/100 g body weight and placed in the stereotaxic instrument (La Précision Cinématographique, Paris). The skull was exposed, the periost removed and two holes (1 mm diameter) drilled above the designated areas. Two pairs of electrodes (stainless steel, 100 μ in diameter, insulated except for the tip) were aseptically implanted in the CA 1 field of the dorsal hippocampus and in the posterior thalamus in planes: L = 4.8; B = 2.0; H = 2.5 mm from the cortex and A = 4.0, L = 0.5; H = 3.0 mm, respectively, according to the stereotaxic atlas [1]. The electrodes were fixed in their position with Acrylic Denture (Simplex, Dental Fillings, London) and the whole implant was anchored on the skull by 8 screws, one of which, above the cerebellum, served as the reference electrode. Another pair of electrodes was attached to the neck muscles to enable recording of the electromyogram (EMG). In 5 animals from each group, a polyethylene cannula was implanted into one of the lateral cerebral ventricles (1.5 mm lateral to the midline and 0.5 mm caudal to bregma) for intracranial application of peptides. Two weeks after surgery, the animals were placed under a 12 hr light/dark schedule (19:00–7:00) in individual sound-attenuating electrically shielded cages with forced ventilation and given food and water ad lib. The electrical activities were transmitted via an impedance transformer (field effect transistors) and cables attached to the electrode plugs on the head of the animal. The first 5 recording sessions (daily from 9:00–17:00 hr of the light cycle) served to determine the sleep-waking pattern, the total amount of PS produced during the recording time and the frequency composition of the theta rhythm prior to treatment. In subsequent sessions, a peptide or placebo (saline) was randomly injected so that two subsequent peptide treatments were separated by a minimum interval of 48 hr. The treatment with each peptide was repeated at least 4 times per animal each serving as its own control. All peptides were freshly dissolved in 10 μl of 0.001 M HCl and further diluted with 0.9% NaCl to the desired concentration. One microliter of the solution was injected into the lateral ventricle. The hippocampal and myographic electrical activities were recorded throughout all sessions and stored on magnetic tape and polygraphic paper for later analysis.

Data Analysis

The amount of PS expressed as a percentage of the total recording time, the number of PS episodes per 60 min and the duration of PS episodes were determined from polygraphic records for each animal and treatment.

To quantify the brain electrical activity the power spectral analysis was used. The hippocampal activity during PS was divided into 10 sec analysis epochs. The activity from each one was filtered (high frequency cut off at 30 c/sec, attenuation 12 dB/octave beyond the cut off), digitalized with a sampling rate of 100 c/sec and the power spectrum computed and stored. Subsequent data reduction in each session involved computation of: (A) averaged spectra per 100 sec of activity; (B) one averaged spectrum per session, based on at least 800 sec of activity; and (C) spectral parameters: mean frequency, peak frequency and spectrum width at 1/2 peak amplitude with corresponding frequencies.

The resolution of 0.1 c/sec of averaged spectra was reduced to 0.5 c/sec per point by applying a window technique in which one point in the new spectrum represented a mean of 5 consecutive points and the resulting spectrum was converted into percentages of power per 0.5 c/sec and stored on paper tape. For subsequent analysis, the mean ± SEM of the percentages of power spectra per animal or group of animals, from the sessions in which the same treatment was given, were computed and plotted against the corresponding mean from placebo treatment sessions. No other peaks appeared in the power spectra in a frequency range exceeding that of the theta band. Consequently, the frequency scale of the illustrations was limited to 0.5–12.0 c/sec. The onset and duration of alterations in hippocampal electrical activity as induced by peptides was examined by analysing the activity in intervals of 0–60, 60–120, 120–180 min and 6 hr after the injection. To simplify the figures, only the data collected in the first two 60 min intervals following treatment were plotted and compared to those after placebo injection. The means of PS percentages, number and duration of PS episodes as well as the means of individual spectral parameters from identically treated animals or groups of animals were compared with corresponding means of controls using Student's t-test.

At the end of the experiment, the position of the cannula tip was marked with Evans blue and that of electrodes by iron deposition for the Prussian blue reaction. The animals were sacrificed by means of an overdose of Nembutal and the brains perfused via the heart with 10% saline formol solution with potassium hexacyanoferrate II (Merck). The placement of the electrode tip was reconstructed on 100 μ thick frozen sections stained with thionine. Only data from those cannulated animals in which the septal area remained undamaged were included in the analysis.

RESULTS

The Influence of Neuropeptides on Quantitative Aspects of PS

Table 1 shows that HO–DI rats generated equal amounts

TABLE 1

RELATIVE AMOUNTS OF TIME OCCUPIED BY SLEEP IN HO—DI AND HO—NO RATS.

	HO—DI N = 7/35	HO—NO N = 6/28	*p*—level
Mean % of PS	12.6 ± 3.1	12.8 ± 2.3	n.s.
Mean % of SWS	54.0 ± 7.5	59.0 ± 7.4	<0.05
Mean number of PS episodes/60 min	2.8 ± 0.6	3.2 ± 0.6	n.s.
Mean duration of PS episode in sec	154 ± 22.2	140 ± 20.5	<0.05

N = Number of animals / number of sessions
± S.D.

of PS compared with HO–NO rats although the PS episodes lasted slightly longer and occurred less frequently than in other groups. The HO–DI animals also spent less time in SWS and thus more time in a waking state. This may be related to a markedly increased need for water by these animals. Intraventricular injections of DG–AVP or ACTH 4–10 did not substantially alter any of the quantitative parameters of PS; neither rabbit serum nor AVP antiserum had any effect (not shown).

The Effect of Neuropeptides on Hippocampal Theta Activity

HO–DI group. Visual inspection of the raw hippocampal records indicated that the rate of theta synchronisation in HO–DI animals was not as high as that of HE–DI or HO–NO animals. A typical segment of the electrical activity during PS in HO–DI and HE–DI rats is shown in Fig. 1. The differences in rhythmicity of hippocampal theta activity between experimental groups became more apparent after statistical evaluation of the spectral parameters in the experimental groups which is given in Table 2. The mean and the peak frequencies of HO–DI animals were 0.6 c/sec lower than those of HE–DI rats. HO–DI rats differed even more markedly from HO–NO animals. Here the difference amounted to 0.8 c/sec for the mean and 0.9 c/sec for the peak frequency. The spectrum widths were comparable in all three groups but the low and the high frequency components which determine the limits of the spectrum width were significantly lower in the HO–DI than in HE–DI or HO–NO rats.

The effect of intraventricularly administered neuropeptides on hippocampal theta rhythm was analysed in 3 out of 5 cannulated animals. The two other rats had not responded to the treatment. In these animals histology revealed partial damage to the septum and a dilatation of the ventricle ipsilateral to the cannula. Injection of 0.02 μg DG–AVP and of 0.06 μg ACTH 4–10 (all L) increased the

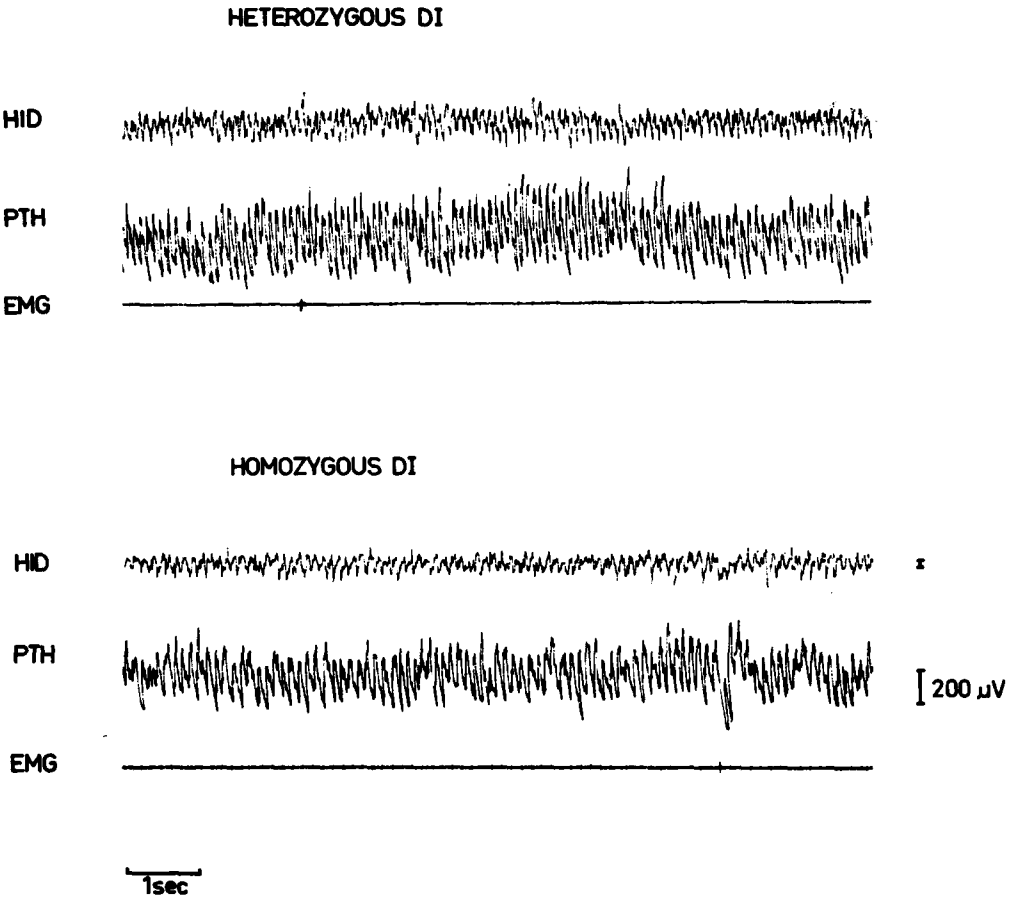

FIG. 1. A segment of theta activity during PS in HE–DI and HO–DI rats. HID = dorsal hippocampus; PTH = posterior thalamus; EMG = electromyogram.

TABLE 2

COMPARISON OF SPECTRAL PARAMETERS OF THETA ACTIVITY DURING PS IN HO—DI, HE—DI AND HO—NO RATS.

	HO—DI N = 8/60	HE—DI N = 7/48	HO—NO N = 6/36
Average mean frequency in Hz	7.3 ± 0.1*	7.9 ± 0.1*	8.1 ± 0.09
Average peak frequency in Hz	6.7 ± 0.2*	7.3 ± 0.2†	7.6 ± 0.3
Average frequencies at 1/2 Peak amplitude	5.5 ± 0.3*–7.4 ± 0.1*	6.5 ± 0.2–8.3 ± 0.3	6.8 ± 0.2–8.7 ± 0.5
Average spectrum width at 1/2 Peak amplitude	1.9 ± 0.4	1.8 ± 0.2	1.9 ± 0.2

N = Number of animals / number of sessions
* = $p<0.001$, from HO—NO
† = $p<0.05$, from HO—NO
± S.D.

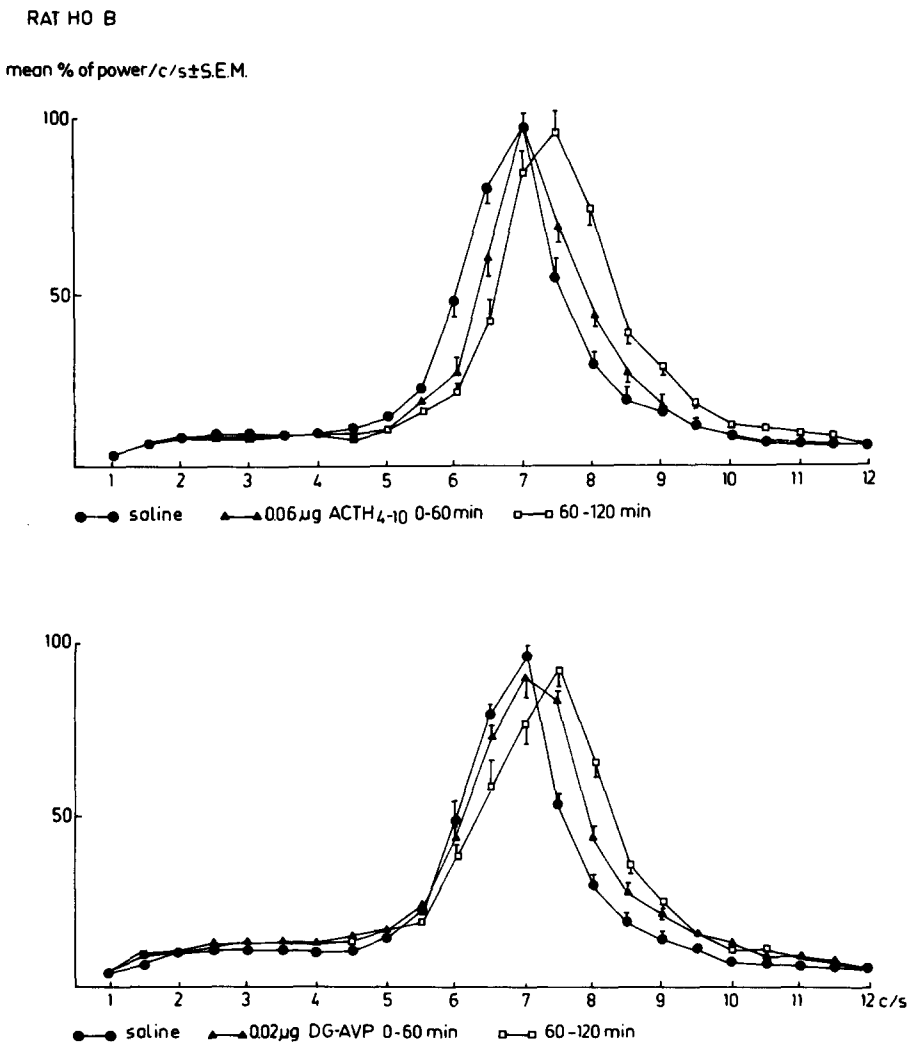

FIG. 2. Frequency composition of theta activity in HO–DI rats 0–60, 60–120 min following intraventricular injection of DG–AVP or ACTH 4–10.

TABLE 3

EFFECT OF TREATMENT WITH ACTH 4–10 AND DG—AVP ON SPECTRAL PARAMETERS OF THETA ACTIVITY IN HO—DI RATS.

	HO—DI N=37/3	HO—DI + $ACTH_{4-10}$ N=8/3	HO—DI + DG—AVP N=9/3
Average mean frequency in Hz	7.3 ± 0.2	8.0 ± 0.1*	7.8 ± 0.2*
Average peak frequency in HZ	6.8 ± 0.2	7.6 ± 0.2*	7.5 ± 0.2*
Average frequencies at 1/2 peak amplitude	5.7 ± 0.3 – 7.7 ± 0.2	6.5 ± 0.1* – 8.6 ± 0.4*	6.3 ± 0.6* – 8.5 ± 0.2*
Average spectrum width at 1/2 peak amplitude	2.0 ± 0.09	2.1 ± 0.2	2.2 ± 0.14

N = Number of sessions / number of animals
* $p<0.001$ from HO—DI
± S.D.

mean frequency of HO–DI animals by 0.5 and 0.7 c/sec, respectively (Table 3). A similar increment was found in the peak frequency. This increased by 0.7 c/sec following DG–AVP and by 0.8 c/sec following ACTH 4–10 administration.

Information about the onset and duration of the effect of the neuropeptides is given in Fig. 3. A slight acceleration of the theta rhythm was observed in the first 60 min after injection of the peptides. More marked alterations followed in the interval between 60 and 120 min after injection. Thereafter, the effects gradually decreased over a period of several hours. With a high dose (0.06 μg) of DG–AVP the changes in electrical activity occasionally persisted for more than 6 hr, however, the activity always returned to the preinjection level within 24 hr.

HE-DI group. An example of the influence of [D-phe^7] ACTH 4–10 as compared to that of ACTH 4–10 on hippocampal theta activity is illustrated in Fig. 3. ACTH 4–10 mildly increased the amount of high frequencies while [D-phe^7] ACTH 4–10 suppressed the high frequencies and stimulated the generation of low theta frequencies in HE–DI rats. The shift of the spectrum to the left as a result of administration of [D-phe^7] ACTH 4–10 was sometimes considerable as shown in an experiment in one HE-DI rat (see Fig. 3). The peak frequency in this animal dropped from 7.5 to 7.0 c/sec after the treatment thus making the theta activity resemble that of HO–DI rats.

Acceleration of the theta activity in HE–DI rats was also seen after administration of 0.02 or 0.04 μg of DG–AVP (not shown).

HO–NO group. The role of vasopressin in the generation of hippocampal theta activity was further studied in normal animals in which the hormone present in the CNS had been inactivated by intraventricularly injected specific AVP antiserum. Interference of 1 μl antiserum with the generation of theta frequencies is illustrated in Fig. 4 which compared the percentages of power per c/sec of HO–NO rats injected with rabbit serum or AVP antiserum with the percentages from HO–DI animals. Note that the frequency pattern in antiserum-treated HO–NO rats is almost identical with that of HO–DI animals.

The onset of the change in theta activity following the injection of vasopressin antiserum had a latency of the order of 60 min and persisted for more than 4 hr. A subsequent test 24 hr later showed a complete recovery of the activity to the preinjection level (not shown). Treatment of HO–NO rats with [D-phe^7] ACTH 4–10 generally did not affect the peak frequency in these animals, but it systematically stimulated the lower and suppressed the higher theta frequencies (not shown).

DISCUSSION

In order to gain more insight in processes underlying the behavioral effects of neuropeptides, the ability of some of these principles to modify hippocampal theta activity and/or the production of PS in Brattleboro rats was examined. The absence of AVP in HO–DI animals had virtually no effect on the amount of PS generated during 8 hr recording sessions (see Table 1). Intraventricular injection of DG–AVP or ACTH 4–10 did not affect the quantity of PS in any of the experimental groups either.

In contrast to the quantity of PS, the hippocampal theta activity during PS was sensitive to changes in intracerebral concentration of AVP, DG–AVP and ACTH 4–10. The inability of HO–DI rats to synthetize AVP was reflected in a marked deceleration of theta rhythm. The mean and peak frequencies in these animals were nearly 1 c/sec below those of homozygous normal animals. The differences in electrical activity between HO–DI and HO–NO or HE–DI rats were limited to frequencies in the theta band since the amount of other frequencies remained comparable among the groups. Thus, the abnormal activity of HO–DI animals during PS cannot be accounted for by either impaired synchronisation or the appearance of additional components but only by the inability of the theta generating substrate to produce fast rhythmicity. Hippocampal activity during other behavioral stages than PS was not quantified. Inspection of EEG records during waking or SWS however did not reveal any patterns different from those of HO–NO animals.

The deviation in the quality of the theta activity of HO–DI rats could be temporarily corrected by DG–AVP, injected subcutaneously [52] or intraventricularly, in quantities which restored the memory deficit of these animals [14]. The increment in peak and mean frequencies was of such magnitude that the frequency composition of the theta rhythm of the treated animals closely resembled

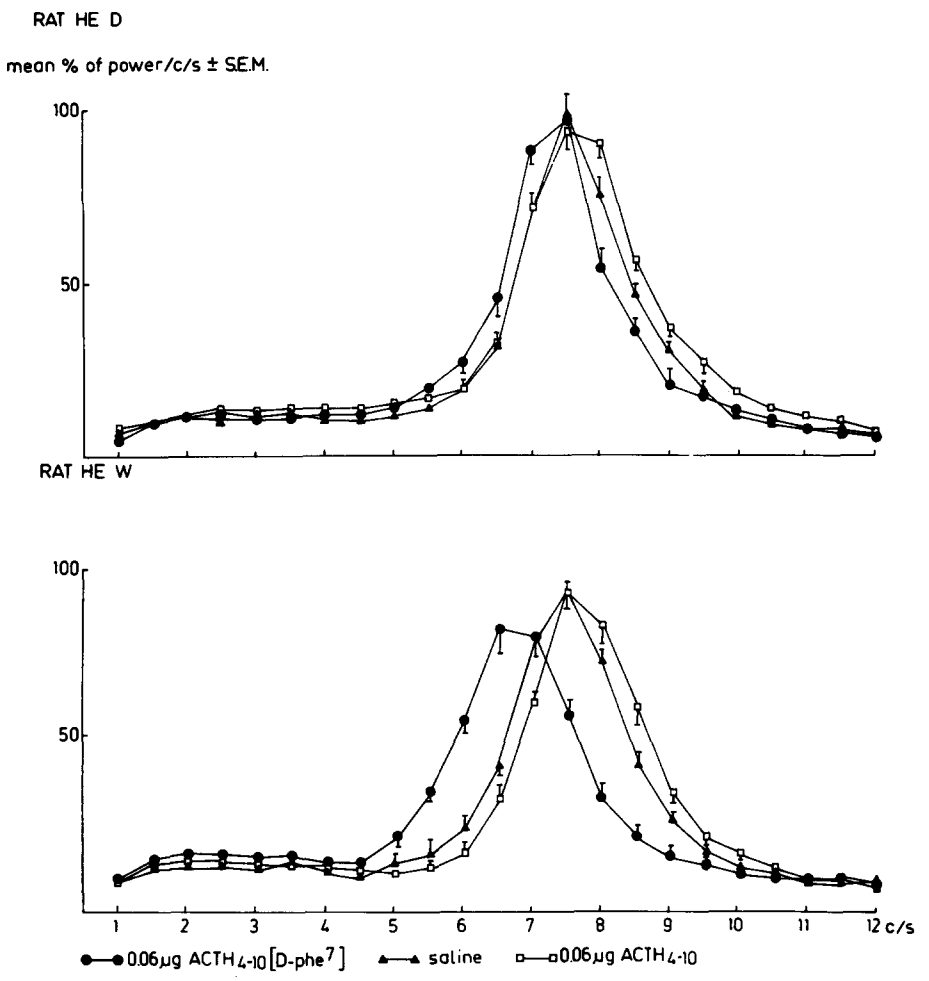

FIG. 3. Effect of ACTH 4–10 and [D-phe^7] ACTH 4–10 on frequency composition of theta activity in HE–DI rats.

that of HE–DI or HO–NO rats (see Tables 2 and 3 for comparison). Other features of hippocampal activity such as the signal amplitude as well as the frequency components outside the range of 4–10 c/sec remained unaffected by DG–AVP (see Fig. 3). ACTH 4–10 was as effective as DG–AVP in normalizing the theta rhythm in HO–DI rats although 3 times as much of ACTH 4–10 was needed to obtain the effect. As a group, HE–DI rats generated slightly less of the high frequency synchronisation than did HO–NO animals (see Table 3). Moses and Miller [38] found that the pituitary of HE–DI rats contained significantly lower amounts of AVP than that of normal rats. This may suggest that the actual amount of available AVP is of importance for the quality of hippocampal activity.

It may be argued that hypothermia or disturbances in the balance of K^+ and Na^+ or alterations in other endocrine functions might be the cause of the abnormal electrical activity in rats which lack vasopressin. However, according to our observations, HO–DI rats do not exhibit a lower core temperature which could explain the low rate of hippocampal theta rhythm [57]. Intraventricular administration of DG–AVP or ACTH 4–10, which normalized hippocampal activity in HO–DI rats, did not affect water intake or urine excretion of these animals (not shown). In addition, DG–AVP is known to be devoid of appreciable antidiuretic and pressor activities [11]. One thus might assume that the treatment had little effect on the hypokalemia and hypernatremia which were found in HO–DI rats [40,41]. Similarly, ACTH 4–10 did not repair the endocrine or metabolic dysfunctions or the physical debilities of hypophysectomized rats [10]. This tends to rule out the possibility that the deviation in brain electrical activity was related to malfunctioning of the pituitary of HO–DI rats [2, 3, 35, 55].

It is conceivable, on the basis of the present data, that vasopressin is involved in the maintenance of normal theta rhythmicity. This is also supported by the experiment in which AVP in the brain of homozygous normal rats has been inactivated by AVP antiserum, a treatment which produced the same rate of hippocampal theta rhythm as that found in HO–DI animals (see Fig. 4). The fact that DG–AVP restored the generation of high frequencies in theta rhythm of HO–DI rats and further stimulated the generation of these frequencies in HE–DI animals is additional evidence that this neurogenic action of vasopressin is dissociated from its endocrine effects.

It has been reported that a subcutaneous injection of ACTH 4–10 enhanced the high frequency components in theta activity induced by electrical stimulation of the mesencephalic reticular formation in freely moving rats [53]. In that study, the effect of the peptide could be mimicked by slightly increasing the stimulus intensity and

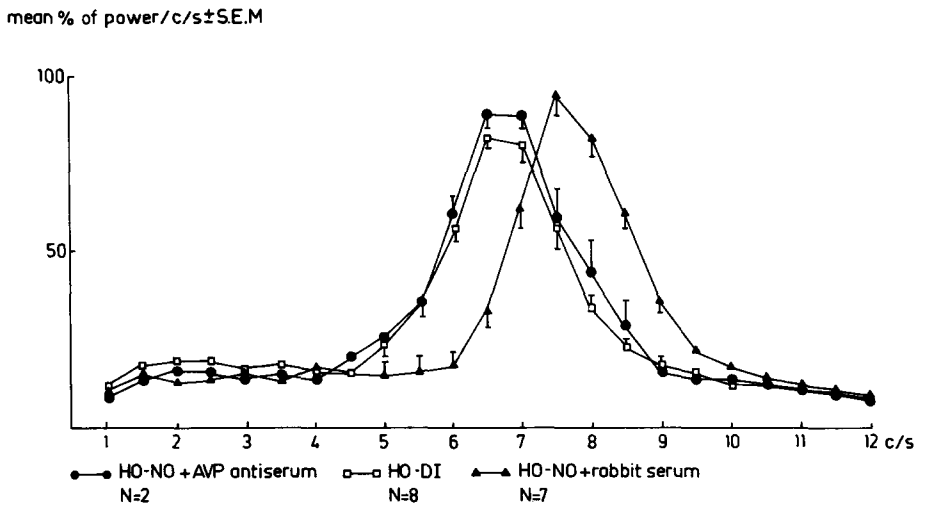

FIG. 4. Frequency composition of theta activity of HO–DI rats, HO–NO rats injected intraventricularly with AVP antiserum and HO–NO rats treated via the same route with rabbit serum.

thus by exciting and engaging more reticular units in the generation of theta rhythm. It is well documented that both the mean and the peak frequency of theta activity are positively correlated with neural activity within mesodiencephalic limbic structures [23,42]. The effects of peptides related to ACTH and AVP on the frequency composition of theta activity may therefore be viewed as a result of an increase (in case of ACTH 4–10 or DG–AVP) or a decrease (in case of [D-phe^7] ACTH 4–10 or AVP antiserum) in excitability of the midbrain limbic network. A specific affinity of these substances towards a particular, functionally or morphologically defined structure however still remains to be demonstrated.

A number of electrophysiological studies with frogs [7,60], rats [44, 45, 46, 49, 50, 52, 53, 58], rabbits [4,24], cats [25, 26, 59], a dog [51] and humans [18, 22, 36] demonstrated changes in brain electrical activity following treatment with ACTH, MSH and related peptides. In most cases these changes could be considered as excitatory. In fact, Steiner [48] has recently demonstrated that ACTH is able to excite the postsynaptic membrane of some hypothalamic neurons. ACTH has also been found to counteract the inhibition of pyramidal cells in the dorsal hippocampus by iontophoretically applied noradrenaline [47]. Similar excitatory properties were shown for oxytocin in rats [39] and for AVP, lysine vasopressin (LVP) and oxytocin in molluscs [5].

The capacity of neuropeptides to alter the excitability of midbrain limbic structures, as measured by changes in the frequency content of hippocampal theta activity, correlated surprisingly well with their effects on avoidance behavior. ACTH 4–10 and DG–AVP increase resistance to extinction of active and passive avoidance behaviors [6,14]. In the present study both peptides enhanced the high frequencies in theta activity. In contrast to ACTH 4–10, treatment with [D-phe^7] ACTH 4–10 or with vasopressin antiserum which facilitates extinction of avoidance behavior [8,56], was found to decrease the amount of high and increase the amount of low frequency components in hippocampal theta activity during PS.

The role of hippocampal theta rhythm in learning and memory is not yet well understood [28] but it is well documented that the quality of this rhythm is closely correlated with the functional state of the midbrain limbic system [42]. The effects of neuropeptides on behavior require the integrity of this system for their manifestation [15]. It is therefore conceivable that the increased excitability along the reticulo-septo-hippocampal pathways found in the present study following administration of neuropeptides may be one of the mechanisms whereby these substances exert their influence on the elaboration and maintenance of conditioned avoidance response.

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