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The Effect of ACTH-Analogues on Motor Behavior and Visual Evoked Responses in Rats

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WOLTHUIS, O. L. AND D. DE WIED. *The effect of ACTH-analogues on motor behavior and visual evoked responses in rats.* PHARMAC. BIOCHEM. BEHAV. 4(3) 273–278, 1976. – Averaged visual evoked responses (VER) in cortical area 17 were recorded one hour after the administration of 7-*l*-phe $ACTH_{4-10}$ or 7-*d*-phe $ACTH_{4-10}$ to artificially ventilated rats, paralysed with gallamine. In addition, the effects of these peptides on spontaneous motor behavior were analysed. The results show that the latencies of all VER components remain unchanged and the amplitudes of the primary VER were unaffected. Measured at a wide variety of light intensities, however, the amplitudes of the VER afterdischarge were significantly and very similarly diminished by both peptides, the effect of 7-*l*-phe $ACTH_{4-10}$ being somewhat stronger than that of 7-*d*-phe $ACTH_{4-10}$. These results support the notion, advanced by others, that these peptides have an effect on a CNS vigilance regulating system, yet do not explain the reported opposite effects on active avoidance behavior of the two related peptides. The effects appear specific since spontaneous motor behavior, as index of changes in generalised arousal, is unaffected by these two peptides.

VER-afterdischarges   Vigilance   ACTH analogues   Spontaneous motor behavior

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ACTH analogues, devoid of corticotrophic activity, restore acquisition of a shuttlebox avoidance response in hypophysectomised rats to normal levels and facilitate (7-*d*-phe $ACTH_{4-10}$) or delay (7-*l*-phe $ACTH_{4-10}$) extinction of active avoidance behavior [6]. These phenomena point at specific effects of these peptides in the central nervous system (CNS). Behavioral evidence suggests that $ACTH_{4-10}$ and related peptides exert their action by affecting attentional or alerting processes [14,21].

Recent electrophysiological results obtained by Urban *et al.* [26,27] suggest that $ACTH_{4-10}$ causes an elevated excitability of the hippocampal theta generating system. Hippocampal theta activity is considered [1,24] to be an EEG concomitant of vigilance and can be elicited by activation of the reticular activating system [9]. Theta activity or shifts thereof are also observed during purposive behavior, e.g. initiation of voluntary movements [29] or transitions in motor behavior [3]; findings which were recently discussed in a review article by Altman *et al.* [2].

An inverse relationship appears to exist between hippocampal theta activity and the afterdischarges or late components of the visual evoked response (VER). Pickenhain and Klingberg [16] demonstrated that VER afterdischarges were blocked during active avoidance responding and more recently they showed [17] that active avoidance responding concurred with hippocampal rhythmic slow activity (RSA or theta). Under appetitive and nonappetitive test conditions Pond and Schwartzbaum [18] and Schwartzbaum and Kreinick [22] showed that increases in behavioral reactivity are associated with suppression of late VER components and with shifts of hippocampal slow activity toward higher frequencies, whereas habituation of behavioral reactivity to flashes was associated with elaboration of late VER components and shifts of hippocampal slow activity toward lower frequencies. Recently, Fleming and Bigler [7] demonstrated that in restrained and unrestrained rats VER afterdischarges could only be elicited during hippocampal episodes of large amplitude irregular slow waves and small amplitude irregular waves, but not when the hippocampus displayed rhythmical slow wave activity (RSA or theta). Additional proof for the existence of a relationship between theta activity and VER afterdischarges is provided by the finding that drugs which pharmacologically induce arousal, e.g.

amphetamines, also induced theta activity [13] and suppress VER afterdischarges [8]. Accordingly, VER afterdischarges are also suppressed when arousal is induced behaviorally [20].

Since the presently studied peptides affect theta generating systems and influence behavioural alertness or vigilance, and since both theta and increased vigilance are associated with attenuated VER afterdischarges, it was interesting to investigate whether and in what way these peptides would alter VER afterdischarges. Because generalised arousal is usually accompanied by changes in activity, we also analysed the effects of both peptides on spontaneous motor behaviour. A priori, great changes in spontaneous motor behaviour (as indices of generalised arousal) were unlikely, since ambulation, rearing, grooming and the number of fecal boli were not or only slightly affected by $ACTH_{1-10}$ in an open field situation [4, 11, 28].

METHOD

Animals

Male small Wistar (WAG) rats of 190–210 g bodyweight were used in all experiments. They were obtained from the breeding centre in the laboratory, where they had been reared under SPF conditions and a 12 hr day–night schedule changing 7 a.m. and 7 p.m.

Drugs

The peptides, 7-*l*-phe $ACTH_{4-10}$ and 7-*d*-phe $ACTH_{4-10}$, were obtained through the courtesy of the Scientific Development Group Organon, Oss, The Netherlands. They were dissolved in glass distilled water and subcutaneously injected in a volume of 1 ml/kg bodyweight. Only one dose level was used in all experiments: 50 μg/kg. Control animals received the same volume of glass distilled water.

Measurements of Spontaneous Behaviour

The measuring apparatus has been described in detail elsewhere [31]. Briefly, it consists of a glass cage placed between the two plates of a capacitor. By applying multilevel detection of movements in a homogeneous electric field the motions of the animals can be categorised quantitatively according to their amplitudes, irrespective of their position in the cage. By employing 4 of these systems motor behaviour of 4 rats can be individually and simultaneously measured. The device has a papertape output. The tape is processed by a PDP8/I computer, using a specially designed Fortran II program. The computer is coupled to a Tektronix 4601 hard copy unit, which produces graphs essentially identical to those in Fig. 1.

Procedure

One hour after the injection each animal was randomly assigned to an activity cage. Four animals were measured simultaneously during 90 min.

Measurements of Evoked Potentials

One day prior to the measurements cortical electrodes were inserted under sodium hexobarbital anesthesia. A hole was drilled in the skull, its center 3 mm to the left of the midline and 3 mm in front of the suture between parietal and interparietal bones, i.e. in the area overlying area 17 according to the atlas of Krieg [12]. The dura mater was left intact. One electrode was inserted in the hole and an indifferent electrode was placed on the bone of the skull immediately above the attachment of the left ear. Both were Ag-AgCl electrodes with a diameter of 0.75 mm. They were insulated and attached to the skull with Sevriton[R] dental cement (De Trey Frères, Zürich, Switzerland); only the tips of the electrodes remained free. In a few preliminary experiments a third electrode was secured in the same way near the nasal corner of the right eye to measure the electroretinogram (ERG). In the latter experiments the other electrode was connected by a fluid bridge of 0.9% NaCl solution. The following day, when the animals had been adapted to total darkness for at least 12 hr, the rats were subcutaneously injected with one of the peptides or the solvent. Thirty minutes later this was followed by an intraperitoneal injection of 100 mg/kg gallamine triiodide (Flaxedil[R], SPECIA, Paris, France). When paralysis set in, the animals were intubated with a polythene cannula and connected to a respiration pump. By infrared analysis of end-tidal CO_2 it had been previously determined [30] that ventilation was adequate. The head was gently fixed in a head holder in such a way that the plane through interaural line and upper incisor bar made an angle of 20° with a horizontal plane, nose down. The pupil of the right eye was dilated with 1% atropine sulphate in 0.9% NaCl. The left eye was covered. All procedures were carried out in dark and light (Kodak Wratten No. 2 filters).

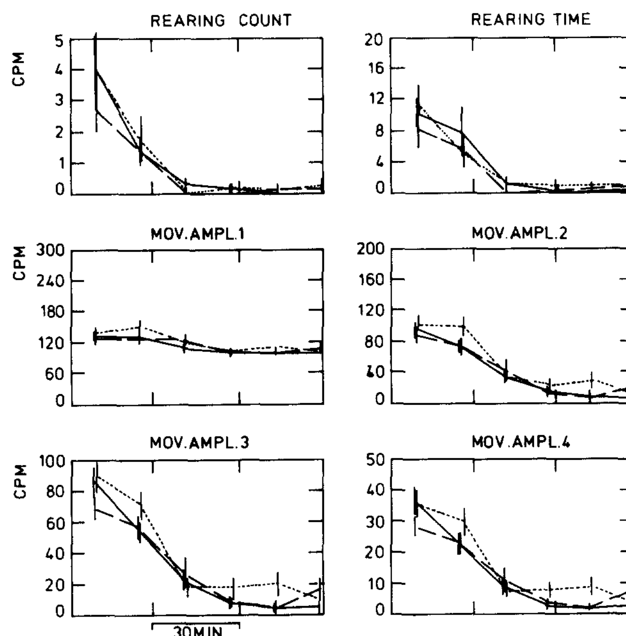

FIG. 1. The (lack of) effects of 7-*l*-phe $ACTH_{4-10}$ (dashed lines) and 7-*d*-phe $ACTH_{4-10}$ (dotted lines) on spontaneous motor behavior in comparison with control rats (drawn lines). Each line represents the mean ± SEM) activities of 6 rats. The top left graph shows the number of rearings per min (cpm) averaged during 16 min periods, the top right graph the time spent in an upright posture, expressed as a percentage of those 16 min periods. The lower graphs represent horizontal movements of increasing amplitudes, movement amplitude 4 being the largest.

The light source was a light emitting diode (LED, Monsanto Type MV2) emitting monochromatic light at 560 nm., i.e. well within the spectral sensitivity of the eye of the albino rat [23]. The advantage of this way of light flash administration is that the amount of light emitted is linearly related to the current through the LED; in this case 3×10^{-9} lumensec was delivered for each mA. The LED was placed at a distance of approximately 3/4 cm of the right eye. The axis through the light source pointed downwards at an angle of 10° with the horizontal plane through the eye and at an angle of 45° with the sagittal plane.

After proper amplification and filtering (high and low frequency cutoff 120 c/s and 0.35 c/s respectively), the signals were visualized on a Tektronix 5103 N storage oscilloscope with 5A22N plug-in amplifiers and stored by a Hewlett Packard 3960 series instrumentation tape recorder. All cortical events following the flash during 500 msec were recorded. Averaging of 100 cortical recordings per animal at each light intensity took place by a Focal program on a PDP8/I computer. In addition to a hard copy representation of the averaged evoked response a numerical printout was made of all averaged peak latencies and amplitudes, so that the means ± S.E.M. per group of identically treated animals could be calculated. This was performed at four light intensities, i.e. 0.3, 2.4, 19.2 and 150×10^{-9} lumensec. Finally, overall averages were obtained by computer averaging the averaged curves of all 6 identically treated rats per group for each light intensity used.

Procedure

From the moment that the animal was experimentally set up, i.e. 35–40 min after the SC injection of the peptides or the solvent and 5–10 min after the IP injection of Flaxedil^R, light flashes were administered at a frequency of 1 per 20 sec at a light intensity of 0.3×10^{-9} each of 2 msec duration were administered. The interflash interval was 2 sec ± 20% random. In 12 blocks, the light intensity was systematically increased from one block to another by a factor of 2.

RESULTS

Electroretinogram

Preliminary results showed no differences between the ERG's of animals injected with the peptides or the solvent, therefore the measurements of the ERG were not further pursued.

Spontaneous Motor Behavior

The results of the measurements of spontaneous motor behavior in 3 groups of 6 rats each are depicted in Fig. 1. The upper left graph shows that the number of rearings/min, averaged over periods of 16 min, are not significantly different between the three groups. Neither is the average time spent in an upright posture, which is expressed as a percentage of those 16 min periods in the upper right graph. Also, no significant difference were observed in the averaged horizontal movements of the 3 groups, represented in the 4 lower graphs. Roughly categorised [31], these graphs represent the following movements: amplitude class 1 is mostly breathing, amplitude class 2 represents small movements of head (e.g. sniffing) or extremities, amplitude class 3 includes extensive movements made by rats remaining in one place (e.g. scratching and grooming) and amplitude class 4 reflects the capacitance changes which occur when the animal is walking. No point in any of the curves differs significantly from any comparable point in the other two curves.

The outcome of a second experiment, also carried out with 3 groups of 6 rats each, was equally negative. In this experiment the animals were injected with the same substances at the same dose levels, but injection took place 16 min after the activity measurements had started instead of 1 hr prior to the measurements.

Visual Evoked Response

In contrast to the absence of any effect on motor behavior, some clearcut effects of the peptides on the VER were demonstrable. In Fig. 2 the overall averages are shown. Only small differences exist between the traces obtained from animals injected with either of the peptides, whereas both peptides clearly suppress VER afterdischarges considerably when compared with the traces from control animals. At four light intensities, i.e. at 0.3, 2.4, 19.2 and 150×10^{-9} lumensec, the latencies and the amplitudes of the VER components were analysed in more detail, the results of which are represented in Fig. 3. The VER components were identified in the same way as used by Creel *et al.* [5], as shown in the bottom right set of traces in Fig. 2. The designations P_2 and N_2 were omitted; these small peaks were not present in the overall averaged traces. It was found that the peptides do not significantly affect the latencies of any of the peaks. By analysis of variances [24] followed by Scheffé's F-test [15] the following differences between treatments were found to be significant; N_1-P_3, saline versus 7-*l*-phe $ACTH_{4-10}$ at the 1% and 7-*d*-phe $ACTH_{4-10}$ versus 7-*l*-phe $ACTH_{4-10}$ at the 5% level. The peak to peak differences N_3-P_3; saline versus 7-*d*-phe $ACTH_{4-10}$ at the 5% and saline versus 7-*l*-phe $ACTH_{4-10}$ at the 1% level of significance. Finally, the differences between N_3-P_4; both saline versus 7-*d*-phe $ACTH_{4-10}$ as well as saline versus 7-*l*-phe $ACTH_{4-10}$ at the 1% level of significance. For the analysis of the heteroscedastic data obtained for N_1-P_1 the weighted analysis of variance proposed by Snedecor [24] was applied.

DISCUSSION

The present results demonstrate firstly, that both peptides have an effect in the same direction, i.e. they both suppress the late VER components and secondly that the effects of 7-*d*-phe $ACTH_{4-10}$ are weaker than those of 7-*l*-phe $ACTH_{4-10}$. Thirdly that these peptides do not significantly affect the amplitudes of the primary response or the latencies of any of the peaks of the VER, and fourthly that these peptides do not influence spontaneous motor behavior.

The findings that both peptides have an effect in the same direction and also that the *d*-phe isomer has a weaker effect are consistent with the observations of Urban *et al.* [26,27] who found that both peptides influenced theta activity in the same direction, the effect of the *d*-phe isomer being smaller than that of the *l*-phe isomer.

Although the results of both studies, i.e. the present one and that of Urban *et al.* [26,27] are clearcut, they offer no explanation for the opposite behavioural effects of the two peptides, shown by Bohus and De Wied [4].

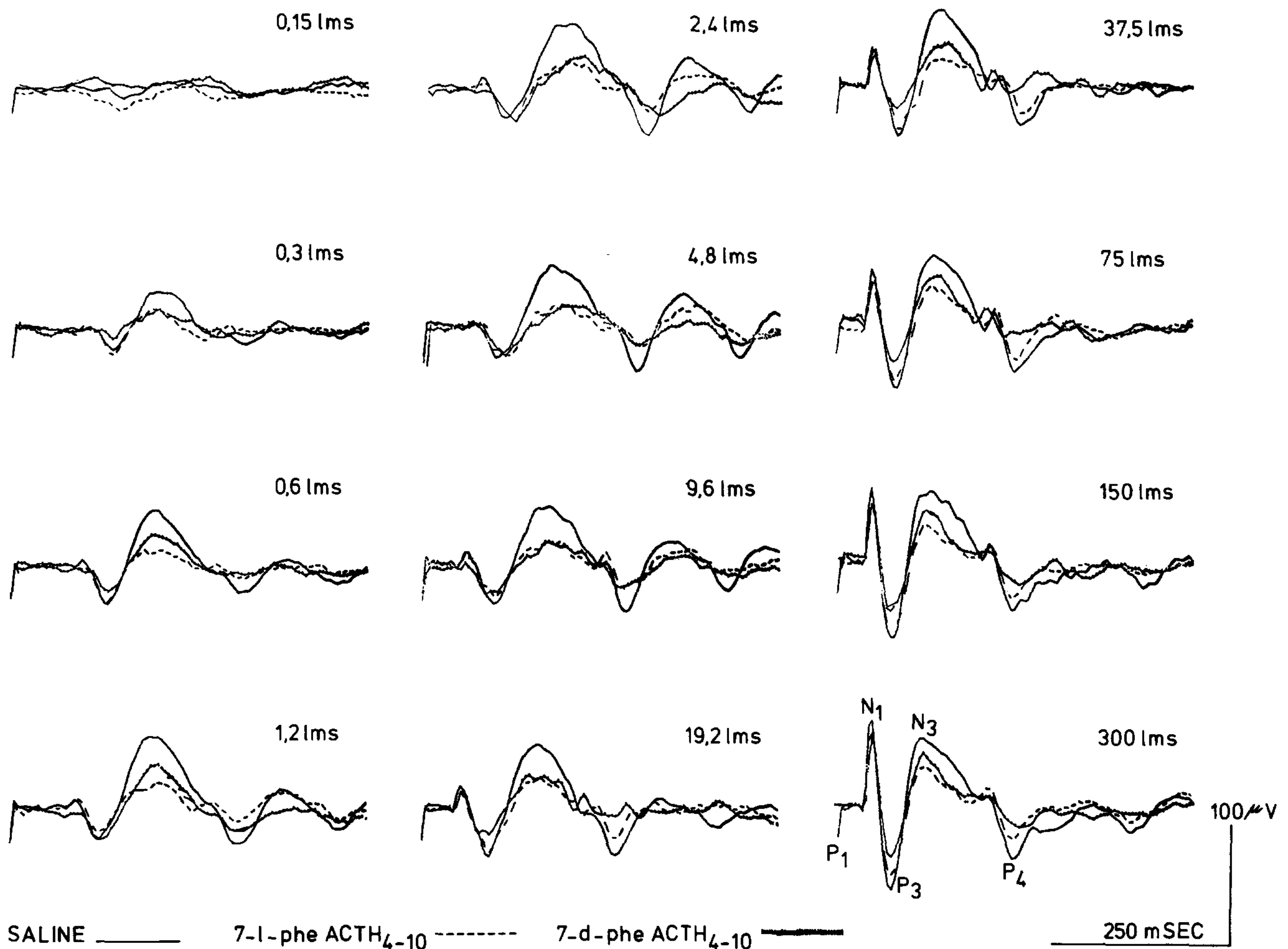

FIG. 2. The effects of 7-*l*-phe $ACTH_{4-10}$ and 7-*d*-phe $ACTH_{4-10}$ on the averaged VER at increasing light intensities, expressed in lumensec (lms). Each trace represents the overall computer-average of the individually averaged VERs of 6 identically treated rats. Compared with the traces obtained from control animals, both peptides suppress the late VER components.

If it is accepted that especially the late components of the VER bear a relationship to behavioural events [19] and if we confine ourselves to a possible explanation for the effects of 7-*l*-phe ACTH (= $ACTH_{4-10}$), then the present data support the existing electrophysiological [26,27] and behavioural [14,21] data, consistent with the notion that $ACTH_{4-10}$ influences systems involved in the regulation of such ill-defined behavioural states as vigilance, alertness, attentiveness or arousal. The results obtained with 7-*d*-phe $ACTH_{4-10}$, however, do not fit into this picture. This may, for example, mean that 7-*d*-phe $ACTH_{4-10}$ in addition affects other brain structures which overrule, reverse or redirect the hitherto investigated phenomena, the net results being a reversal of behaviourally demonstrable effects. Alternatively, it may be that the two peptides have opposite effects on an entirely different CNS structure, which are correlated with the behavioural effects whilst they have an effect in the same direction on the CNS vigilance regulatory system.

Whatever the explanation may be, the suppression of the VER afterdischarges cannot be explained by generalised behavioural arousal caused by the peptides. Other pharmacological agents, such as sympathicomimetics (amphetamine, metamphetamine) which are known to cause more generalised arousal, do not only suppress VER afterdischarges [8], but also have profound effects on spontaneous motor behaviour [10,31]. The two peptides tested here, however, have no effects on spontaneous motor behaviour at all.

If one considers the rapid decline in spontaneous motor activity which occurs both in control and in experimental rats (see Fig. 1) as an expression of fast habituation to what must be a rather dull environment (glass cage), then it can be concluded that both peptides have no effect on this type of habituation.

The conclusions from the present experiments can be summarised as follows. Judged by their effects on the VER-afterdischarges, the present results support the notion that both peptides have an activating effect on a vigilance regulating system. The effect of 7-*d*-phe $ACTH_{4-10}$ is

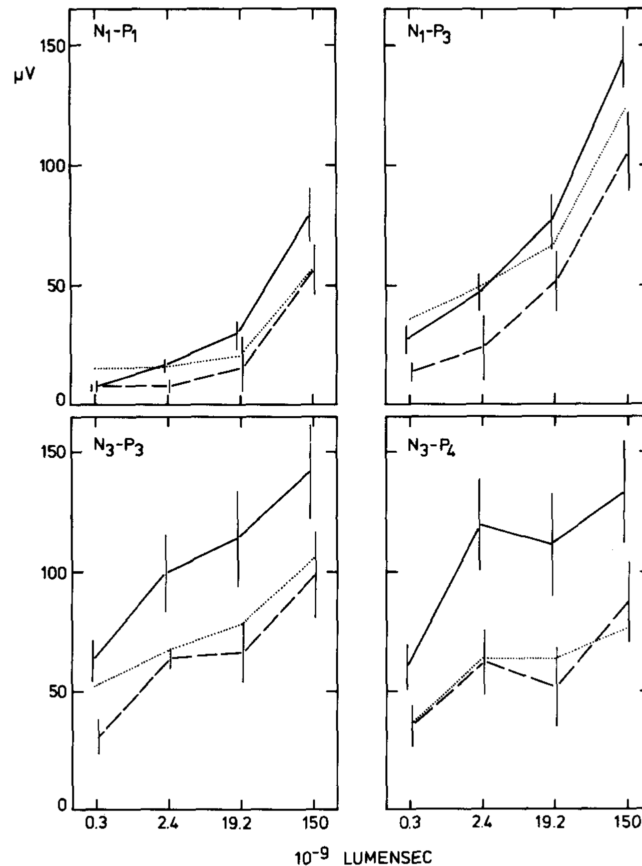

FIG. 3. Peak to peak amplitudes of the various VER components measured at four light intensities. While 7-*l*-phe $ACTH_{4-10}$ (dashed lines) or 7-*d*-phe $ACTH_{4-10}$ (dotted lines) do not or slightly affect the amplitude differences of the primary response, they significantly suppress the amplitude differences of the late VER components. For the sake of clarity the vertical bars (SEM) are only drawn for two of the three curves, those omitted are of similar order of magnitude.

somewhat weaker than that of 7-*l*-phe $ACTH_{4-10}$. The data indicate that both peptides have effects in the same direction. Therefore, the present findings do not offer an explanation for the opposite behavioural effects of the two peptides. The suggested increase in vigilance caused by both peptides seems specific, since spontaneous motor behaviour, as an index of more generalised arousal is not affected by either of the two peptides.

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