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Effects of an ACTH 4–9 Analog on Auditory Evoked Brainstem Responses and Middle Latency Responses

J. BORN, G. FEHM-WOLFSDORF, D. J. NAGEL, K. H. VOIGT AND H. L. FEHM

Abtlg. Innere Medizin I, Abtlg. Physiologie, Abtlg. HNO-Heilkunde
Universität Ulm, Parkstr. 11, 7900 Ulm, F.R.G.

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BORN, J., G. FEHM-WOLFSDORF, D. J. NAGEL, K. H. VOIGT AND H. L. FEHM. *Effects of an ACTH 4–9 analog on auditory evoked brainstem responses and middle latency responses.* PHARMACOL BIOCHEM BEHAV **23**(3) 367–372, 1985.—Early and middle latency auditory evoked potentials (EAEPs and MAEPs) were recorded from thirteen male volunteers after oral administration of either 40 mg of an ACTH 4–9 analog (ORG 2766) or placebo. Main results indicate slightly longer latencies of the later components of the EAEPs after ACTH 4–9 analog. Effects of differences in treatment were clearest with very high stimulus rates. Therefore, these effects do not lend themselves for the explanation of ACTH 4–9 analog-induced changes in long latency auditory evoked potentials of cortical origin obtained with comparatively slow stimulus rates in earlier studies. In addition, the ACTH 4–9 analog inhibited a decrease in amplitudes of the Na component of the MAEP across the session. This latter result may be in line with dishabituating actions of the peptide.

Early latency auditory evoked potentials (auditory evoked brainstem responses) Middle latency auditory evoked potentials ACTH ACTH 4–9 analog

IN humans, peripherally administered fragments of the adrenocorticotropic hormone (ACTH) were found to alter cortical activity as indicated by spontaneous EEG activity and auditory evoked responses [1, 5, 6]. ACTH-fragments presumably act on receptors in the area postrema: the responses to peripherally administered ACTH 4–10 of dopaminergic neurons in the nucleus arcuatus of the hypothalamus and in other extrahypothalamic structures in rats were abolished following lesions of this structure [12]. It is supposed that septal and hippocampal regions are essential for behavioral effects of ACTH-fragments [4, 22, 23]. MET-(O2)-GLU-HIS-PHE-D-LYS-PHE-OH, a highly potent analog of ACTH 4–9, was found to alter long-term habituation after oral intake of this substance in man [1,15]. These effects were confirmed by related changes in cortically generated components of the auditory evoked response and were assumed to be mediated via structures of the limbic system.

It was of interest to see whether the effects of the ACTH 4–9 analog on evoked responses of cortical origin are mediated by lower brainstem and thalamic centers as reflected in an effect on earlier evoked responses. The present experiment was designed to investigate the influences of this peptide on early auditory evoked potentials (EAEPs) and on middle latency responses (MAEPs). Whereas EAEPs are known to be generated in the brainstem nuclei [2,10], components of the MAEPs may be of thalamic or of early cortical origin [8, 11, 16]. Effects of the ACTH 4–9 analog on the brainstem nuclei may cause changes in stages of sensory information processing subsequent to the EAEPs, i.e., in cortically generated evoked responses of longer latencies observed in an earlier study [1]. In addition, peptide-induced changes in brainstem evoked responses could question previously proposed interpretations of effects of the ACTH 4–9 analog in terms of habituation, because EAEPs do not habituate and appear to be rather unaffected by fluctuations of central nervous arousal [7, 14, 16, 17].

METHOD

Subjects

Thirteen male adults (normally hearing, non-smokers, aged 18–30 years) voluntarily participated in the experiment. They were not under current medication and had to abstain from coffee and alcoholic beverages for at least twelve hours prior to the experimental sessions.

Design and Procedure

Subjects were tested according to a double-blind crossover design. They received either 40 mg of an ACTH 4–9 analog (ORG 2766) or placebo orally one hour prior to testing. The two sessions were 14 days apart. They were scheduled at 3:00 p.m. and lasted for about 150 minutes.

Testing took place in a sound attenuated and electromagnetically shielded booth. The subject lay on a couch, relaxed but with his eyes open. Stimuli were filtered (at 2000 Hz) rarefaction clicks (duration: 0.25 msec) presented binaurally in order to assure reliable identification of EAEP components even with low click intensities. Presentation of the series of clicks for EAEP- (1000 clicks) and MAEP-averaging (300 clicks) was balanced across subjects for each of three

TABLE 1
EXPERIMENTAL PROCEDURE

3:00 p.m.	drug intake: ACTH 4–9 analog vs. placebo			
	condition	series	response measured	intensity, stimulus rate
4:00 p.m.	persistent stimulation			
	(1. block)	1–4	EAEP	80 dB HL, 10/sec
		1–4	MAEP	80 dB HL, 4/sec
	(2. block)	1–4	MAEP	80 dB HL, 4/sec
		1–4	EAEP	80 dB HL, 10/sec
4:45 p.m.	low intensity	1–2	EAEP	50 dB HL, 10/sec
		1–2	MAEP	50 dB HL, 4/sec
5:00 p.m.	stimulus rate	1	(baseline)MAEP	80 dB HL, 4/sec
		2–3	MAEP	80 dB HL, 10/sec
		1	(baseline)EAEP	80 dB HL, 10/sec
		2–3	EAEP	80 dB HL, 50/sec
5:20 p.m.	control series: including 0 dB HL averaging and click presentation under applied muscle tension			
5:30 p.m.	post-experimental ratings of perceived physiological changes			

In the left column it is indicated at about what time conditions started. The order of EAEP and MAEP series was balanced for each condition across subjects. Also, the baseline series of the 'stimulus rate' condition could be either the first or the third, balanced across subjects.

stimulus conditions (Table 1). In the first, 'persistent stimulation' condition, 80 dB HL clicks were presented at a rate of 10/sec (EAEPs) or 4/sec (MAEPs). For the subsequent series of clicks the intensity was lowered to 50 dB HL ('intensity' condition). In a third condition, the effects of a faster stimulus rate (for EAEPs: 50/sec; for MAEPs: 10/sec) were tested in two series of clicks (80 dB HL). This 'stimulus rate' condition, in addition, included a series of clicks presented at the baseline stimulus rate, which was used in the first, persistent stimulation condition. These series were either the first or the last of this condition, balanced across subjects.

Final control recordings using 0 dB HL stimuli and stimulus presentation under conditions of applied muscle tension, yielded no consistent, time-locked EAEPs or MAEPs. At the end of the session, the subject rated perceived physiological changes (e.g., in heart rate) and feelings of tiredness on seven point rating scales. He also had to report whether he assumed to have received placebo or an active agent.

Recording and Apparatus

Recordings of the EEG were obtained from the vertex (Cz) referenced to the left mastoid, with the ground at Fpz. For EAEP recording the band pass (Krohn-Hite Variable Filter Mod 3750) was set at 0.1–6 kHz (24 dB/oct), and at 0.002–6 kHz for MAEPs. Further amplification, digitization, averaging, and the delivery of clicks was provided by an averaging system (Madson ERA 2250). An artifact rejection excluded sweeps containing high amplitude voltage activity. Sweep times were either 10 msec (EAEPs) or 50 msec (MAEPs), and sampling of 250 data points per sweep started synchronously with stimulus onset. The averaged waveforms were displayed on a monitor and plotted on an XY-plotter.

Data Reduction and Analysis

Measurements of the amplitudes and latencies of the evoked response components were made in a blind manner with respect to the treatment. Peaks of the EAEPs (I-VI, vertex positive, and the corresponding negative troughs, I_n-VI_n) and MAEPs (Po, Na, Pa) were identified visually. Latencies were calculated with reference to stimulus onset. The determination of amplitudes for EAEPs was limited to the most prominent component V, which was measured as the difference between this and the following V_n. Amplitude differences were also calculated between the Na and Pa components of the MAEPs and between wave V and the Na. The Po-amplitude was calculated with reference to the relatively constant amplitude of wave V, and in addition, with reference to the successive Na.

The evaluation of experimental effects was based on within-subject comparisons. ANOVAs including repeated measures were applied to the data of each of the three conditions in order to test for treatment and condition effects. To evaluate slower changes across the session an additional ANOVA was performed on a set of data from the first series of each block of the persistent stimulation condition and from the baseline series of the stimulus rate condition.

The series of low intensity clicks and those of high stimulus rate were analysed for wave V, V_n, the Na and Pa, only. Wave IV_n was not analysed and the analyses of wave IV and the Po-component was based on a smaller subject

TABLE 2

MEAN LATENCIES (LAT.) AND AMPLITUDES (AMP.) OF THE EAEP (UPPER PANEL) AND MAEP (LOWER PANEL) COMPONENTS (WAVE I-VI$_n$, Po, Na, AND Pa) FOR THE THREE EXPERIMENTAL CONDITIONS (PERSISTENT STIMULATION, LOW INTENSITY STIMULATION, STIMULUS RATE) AND BOTH TREATMENTS (ACTH 4–9 ANALOG VS. PLACEBO)

Condition	Persistent Stimulation					Low Intensity			Stimulus Rate (Baseline)					
	Block I		Block II											
FAEPs	10/sec-80 dB HL					10/sec-50 dB HL			10/sec-80 dB HL			50/sec-80 dB HL		
	ACTH 4–9	Placebo	ACTH 4–9	Placebo	SD	ACTH 4–9	Placebo	SD	ACTH 4–9	Placebo	SD	ACTH 4–9	Placebo	SD
I lat.	1.89	1.88	1.88	1.91	(0.17)				1.92	1.85	(0.18)			
I_n lat.	2.50	2.48	2.52	2.51	(0.22)				2.52	2.53	(0.24)			
II lat.	3.07	3.08	3.10	3.10	(0.28)				3.11	3.07	(0.29)			
II_n lat.	3.49	3.52	3.52	3.51	(0.22)				3.49	3.45	(0.25)			
III lat.	4.02	4.00	4.03	4.02	(0.16)				4.03	4.01	(0.15)			
III_n lat.	4.58	4.57	4.61	4.59	(0.23)				4.67	4.61	(0.26)			
IV lat.	5.24	5.18	5.25	5.20	(0.15)				5.26	5.27	(0.13)			
V lat.	5.89	5.85	5.91	5.90	(0.19)	7.26	7.30	(0.36)	5.89	5.93	(0.23)	6.28	6.21	(0.23)
V_n lat.	6.66	6.64	6.69	6.69	(0.19)	8.21	8.27	(0.33)	6.74	6.71	(0.17)	7.28	7.16	(0.25)
VI lat.	7.62	7.54	7.65	7.64	(0.24)				7.74	7.65	(0.20)			
VI_n lat.	8.17	8.10	8.23	8.19	(0.33)				8.27	8.27	(0.30)			
V–V_n amp.	0.516	0.526	0.511	0.502	(0.121)	0.239	0.260	(0.084)	0.502	0.495	(0.132)	0.425	0.411	(0.123)
	Block I		Block II											
MAEPs	4/sec-80 dB HL					4/sec-50 dB HL			4/sec-80 dB HL			10/sec-80 dB HL		
Po lat.	12.96	12.82	12.49	12.92	(1.28)				12.52	12.61	(1.33)			
Na lat.	18.91	18.97	19.07	19.24	(2.14)	20.47	20.24	(2.32)	19.93	20.09	(3.45)	19.18	19.07	(2.44)
Pa lat.	30.52	31.15	30.67	30.78	(2.61)	31.78	31.07	(2.94)	30.23	29.44	(3.75)	31.12	30.62	(3.77)
V–Po amp.	0.390	0.463	0.300	0.502	(0.441)				0.374	0.368	(0.489)			
Po–Na amp.	0.939	0.946	0.944	0.877	(0.485)				0.832	0.749	(0.480)			
V–Na amp.	1.33	1.40	1.33	1.32	(0.41)	1.06	1.10	(0.54)	1.38	1.17	(0.48)	1.30	1.33	(0.48)
Pa–Na amp.	1.26	1.34	1.27	1.32	(0.48)	1.05	1.11	(0.46)	1.34	0.89	(0.41)	1.29	1.31	(0.47)

Latencies are given in msec and amplitudes in μV. The average standard deviations (SD) for each condition are shown in parentheses.

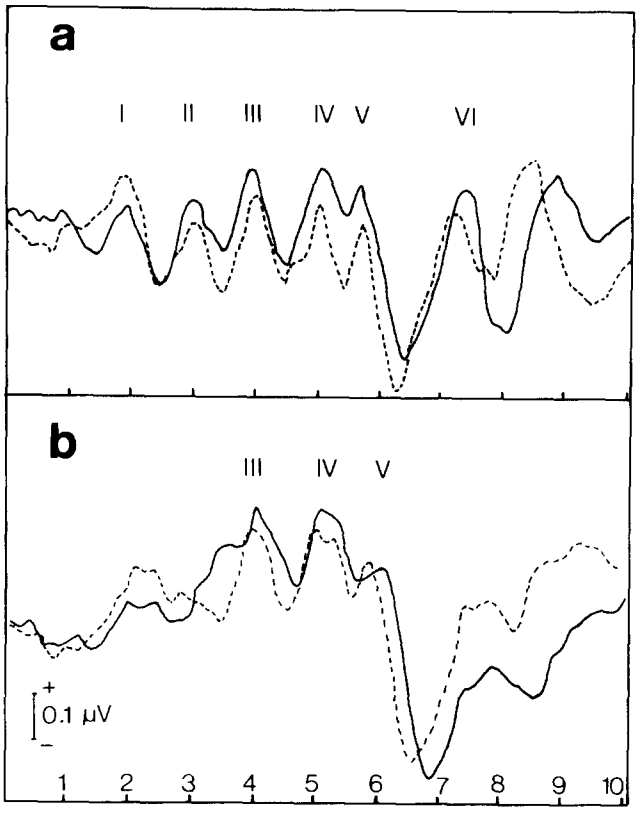

FIG. 1. EAEPs of a single subject (a) under the condition of baseline rate of click stimulation (10/sec), (b) under speeded delivery of clicks (50/sec) for the placebo (dotted line) and ACTH 4–9 analog session (solid line). The latencies of the wave V and the subsequent negative V_n peak are delayed by the increased rate of stimulation, and this is more evident after ACTH 4–9 analog. Vertex positive upward. The scaling of the x-axis is in msec.

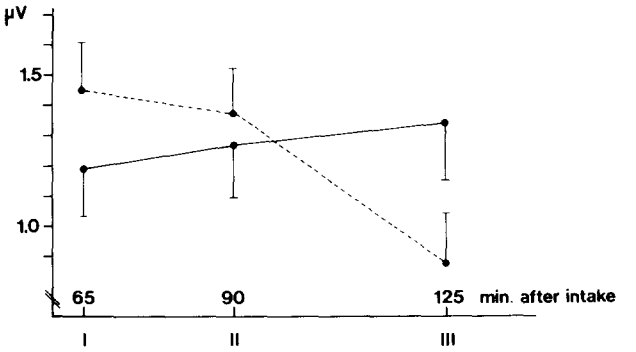

FIG. 2. Average amplitudes of the Pa-Na difference of the MAEPs for the first series of the two blocks of the persistent stimulation condition (I, II) and the baseline series of the stimulus rate condition (III). Following the administration of ACTH 4–9 analog (solid line) the decrease of the amplitude towards the end of the session, which can be observed after placebo (dotted line), is inhibited.

sample since these components could not be reliably identified in all subjects. The post-experimental ratings were evaluated by *t*-tests.

RESULTS

After the ACTH 4–9 analog, subjects felt somewhat more excited during the experiment, $t(12)=2.70$, $p<0.02$. Except for this effect, the post-experimental ratings did not differ between treatments. Eight of 13 subjects correctly identified their order of treatment, which was not significantly more than by chance (Binomial test: $p(13;>8)<0.30$).

Table 2 reviews the mean latencies and amplitudes of the analysed components of the EAEPs and MAEPs with respect to the experimental condition. In the persistent stimulation condition latencies of the EAEP components did not show any significant alterations across the single series. Latencies of the components III_n, V, V_n, VI and VI_n, however, were significantly increased ($F(1,11)=8.36$, 11.01, 21.67, 13.97, and 8.06, respectively, $p<0.05$) towards the second block of four series. Furthermore, a significant increase in latencies for the components III, III_n, V_n, VI, and VI_n ($F(2,22)=3.32$, 4.65, 14.48, 6.03, and 4.82, respectively, $p<0.05$) was visible across the first series of the blocks of the persistent stimulation condition and the baseline series of the stimulus rate condition. Following the administration of the ACTH 4–9 analog, generally enhanced latencies were found for the latest component VI_n of the EAEPs in the persistent stimulation condition, $F(1,11)=4.61$, $p<0.05$. Similar, but statistically insignificant enhancements were visible for some of the earlier waves (III, III_n, V, and VI). For wave IV, treatment differences also reached significance, $F(1,7)=9.4$, $p<0.02$. Amplitudes of the wave $V–V_n$ showed no systematic alterations for this condition.

The lowering of the click intensity led to significantly longer latencies of the components V, $F(1,11)=257.7$, $p<0.001$, and V_n, $F(1,11)=292.0$, $p<0.001$, with no significant differences between treatments. The latencies of these peaks were also increased by an enhanced rate of stimulation (V: $F(2,22)=34.88$, $p<0.001$, V_n: $F(2,22)=60.11$, $p<0.001$). After administration of the ACTH 4–9 analog this increase in latencies was especially noticeable (V: $F(2,22)=4.48$, $p<0.025$, V_n: $F(1,11)=5.71$, $p<0.05$). The $V\text{-}V_n$ amplitude was reduced after low intensity clicks, $F(1,11)=88.03$, $p<0.001$, and also during fast stimulus rates, $F(2,22)=5.09$, $p<0.02$, but remained unaffected by the peptide.

Latencies of the Po and Pa components of the MAEPs did not show any systematic changes related to experimental variations. This negative result may have been due to the large variability of MAEP components. The latency of the Na component was enhanced by reducing the click intensity, $F(1,11)=17.54$, $p<0.002$, but it was not influenced by any other experimental variation.

Amplitudes of the Po did not display any systematic fluctuations. Both of the Na-related amplitude differences, wave V-Na and Pa-Na decreased under low intensity stimulation (V-Na: $F(1,10)=26.86$; Pa-Na: $F(1,9)=49.42$, $p<0.001$). After placebo, but not after the ACTH 4–9 analog, the Pa-Na amplitude decreased across the first series of the blocks of the persistent stimulation condition and the baseline of the stimulus rate condition, $F(2,16)=3.59$, $p<0.05$. For the V-Na difference the effect was not significant.

DISCUSSION

The experiment proved to be useful for an investigation of the effects of the ACTH 4–9 analog on EAEPs and MAEPs on a background of variations of these waveforms induced

by manipulations of the stimulus parameters. The reduced intensity and the enhanced rate of click stimulation led to prolonged latencies of the waves V and V_n of the EAEPs, which is in accord with previous reports [7, 9, 16]. We found a very slight, but significant, increase in latencies of some of the later EAEP components across experimental blocks and across the session. Diurnal oscillations towards a decreasing body temperature during the time of testing may have triggered the observed enhancements [13,18]. The lying position of the subjects may have contributed to the fall in body temperature. Another possibility, suggested by the findings of decreased rates of neuronal firing in auditory brainstem nuclei of cats after repeated presentation of tones [3], is that a direct neuronal inhibition was provoked by a repetitive presentation of stimuli.

Under certain conditions, the ACTH 4–9 analog led to small but significant effects on selected features of the evoked responses. For the slightly longer latencies of the later components of the EAEPs after the ACTH 4–9 analog, treatment-related changes in the tonic activity of the middle ear muscle cannot be ruled out, since fragments of ACTH were found to alter muscle activity [19,20]. However, an action of the peptide on the neuronal paths seems more likely, since myogenic changes would be expected to mediate changes of all EAEP components, whereas the effects of the ACTH 4–9 analog were limited to the later EAEP components.

Treatment effects on EAEPs were already present in the beginning of the series of the persistent stimulation condition, but were most prominent under increased rates of stimulation. As differences between the effects of treatments in series of low intensity clicks were not significant, it can be assumed that sensory thresholds are not heavily influenced by the peptide. The observed changes after the ACTH 4–9 analog more likely reflect a heightened sensitivity of the auditory brainstem nuclei to high loads of sensory input after the ACTH 4–9 analog.

The occurrence of pronounced changes in the latencies of the later components of the EAEPs apparently depended on a high stimulus rate. The slow rates of stimulation typically used in investigations of cortically generated potentials, hence, cannot be expected to produce any latency changes at all at the brainstem level of auditory paths after the ACTH 4–9 analog. Therefore, it is unlikely that dishabituating effects of the peptide, which were observed in auditory evoked potentials of longer latencies [1,6], were a consequence of altered EAEPs. On the other hand, brainstem neurons obviously are affected by the peptide. The EAEP reflects neuronal activity that is tightly time-locked to the stimulus. Other response properties of the neurons could be greatly modified before an effect on their synchrony of firing becomes manifest. Very small effects on lower brainstem levels may affect responses at a cortical level owing to divergence and convergence of neuronal networks. Hence, the possibility of a mediation of effects of the ACTH 4–9 analog on auditory evoked cortical responses by lower brainstem centers cannot be completely excluded on the basis of the present findings.

The MAEP findings were obscured by the enhanced variability of these components, which was presumably due to myogenic activity known to contribute to the MAEP components [21]. The Na, which may be an early cortically generated response [8,21], showed—in line with other reports [7]—enhanced latencies and reduced amplitudes with low intensity clicks. Across placebo sessions, the Na-Pa component of the MAEP gradually decreased in amplitude. After the ACTH 4–9 analog, no similar decrease in amplitude could be observed. Long-term habituation of MAEP components has not been sufficiently investigated, but, in general, cortically generated evoked potentials of longer latencies are subjected to habituation as indicated by decreasing amplitudes. Therefore, the inhibited decrease of the Na-Pa amplitude in the presence of the ACTH 4–9 analog may support a dishabituating effect of the substance, which was also found for cortically generated evoked potentials of longer latencies [1].

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