Studies on the Possible Central Effects in Man of a Neuropeptide (ACTH 4–9 Analogue)

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Summary. The central effects of a neuropeptide, ACTH 4–9 analogue (Organon 2766), were studied in man using digit symbol substitution (DSS), symbol copying, digit span, electroencephalography and auditory evoked potentials, critical flicker fusion (CFF) and pupillary response to light. Performance was measured overnight, and each of 6 subjects ingested 300 mg caffeine, 40 mg ACTH 4–9 analogue and matching placebo. With placebo there was a marked deterioration in performance overnight. The number of substitutions on DSS and the numbers of symbols copied fell, and the threshold for CFF and number of errors on the vigilance task increased. These effects were not seen after ingestion of caffeine (300 mg), though caffeine may have led to some deterioration in the ability to remember digits. The neuropeptide did not attenuate the decrements in performance overnight.

Key words: ACTH 4–9 analogue; neuropeptide, performance, caffeine, electroencephalography, central effects

Improvements in performance with ACTH-like peptides have been observed in animals, and attributed to enhancement of selective attention [21], increased arousal in response to motivationally relevant stimuli [23], and beneficial effects on memory consolidation [7, 9]. However, in man there is no consistent evidence of changes in memory or cognition, though the peptide ACTH 4–10 and the orally active synthetic analogue of ACTH 4–9 have been reported to lead to improvements in sustained vigilance [18]. Such compounds may also modify task-related electroencephalographic activity [5].

Previously, we studied the effects of 40–120 mg of the ACTH 4–9 analogue on the nocturnal sleep of healthy man [14], and, unlike caffeine (300 mg) which was used as the active control and other drugs which improve vigilance [15, 16], it failed to increase wakefulness during sleep – at least as measured by standard sleep stage analysis. Nevertheless, in view of reported improvements in vigilance, we have studied the effects of ingestion overnight of the ACTH 4–9 analogue when there is a marked decline in performance.

Methods

Experimental Procedure

The subjects were 6 healthy females aged between 19 and 34 years (mean 25 years) and weighed between 45 and 72 kg (mean 58 kg). They were not involved in any other drug therapy except possibly the use of oral contraceptives, and measurements within 2 days of the onset of menstruation were avoided or repeated. Subjects retired to bed at their usual time the night before the experiment. From 13.30 h on the day before the experiment they avoided alcohol, caffeine containing beverages and unusual exercise, and they refrained from napping.

Performance was tested overnight during two sessions of 2.75 h duration (pre-ingestion and post-ingestion) which began at 21.30 and 00.45 h respectively. Each subject ingested 300 mg caffeine, 40 mg ACTH 4–9 analogue (Organon 2766) or matching placebo at 00.30 h, between the two performance sessions. A random design was used and the study was double-blind. During each of the performance sessions digit symbol substitution (DSS), symbol copying (SC) and digit span (DS) were measured between
and AEP recorded between 140-165 rain.

Performance

Digit Symbol Substitution and Symbol Copying. The subjects were given two sheets, and required to complete as many spaces as possible for each sheet. Errors were very rare, and so the number of substitutions completed within 4 min was used as the performance measure. With symbol copying they were given two sheets and required to copy as many symbols as possible. The number of symbols copied within 2 min was the performance measure.

Digit Span. A 5 digit number was presented on a visual display monitor for 3 s. The subjects were required to commit the number to memory, and after 4 s type the number on a keyboard. If the 5 digits were recalled correctly, a 6 digit number was presented. The number of digits presented increased by one after each correct response, and decreased by one after two consecutive incorrect responses. The maximum number of digits was 14. The task lasted 10–15 min during which 25 numbers were presented. The first five presentations were ignored, and the mean number of digits presented was scored.

Critical Flicker Fusion. This threshold was measured using two visual fields [17].

Visual Vigilance. Random digits (0–9) were displayed on a television screen at a rate of 1/s. Contrast and brightness of the screens were kept constant. The subjects indicated by a push button when 3 consecutive, different odd digits appeared (e.g. in the segment “2–9–6–3–7–1–4–7–8” ... “3–7–1” would be the critical sequence). The task lasted 45 min and there were 119 randomly spaced target sequences. The number of correct, wrong and missed responses were recorded.

Pupillary Diameter. A television pupillometer with an infra-red sensitive camera based on the design of Green and Masseidevag [11] was used. The pupil was illuminated by infra red light reflected from the retina. Pupillary size was recorded for a 40 s control period, followed by the responses to 4 flashes of light of 0.5 s duration from a Maxwellian view light stimulator. Each flash was separated by 10 s, and followed by a recovery period of 1 min [17]. The resting diameter of the pupil and change in response to each flash of light were measured.

Electroencephalography

Electroencephalogram (EEG). Resting activity was recorded using silver-silver chloride electrodes with resistance of less than 10 kΩ. The electrodes were positioned according to the 10–20 international system. The EEG was recorded for 2.5 min between Cz and paired mastoids and between P3 and O1, once with eyes open and once with eyes closed. At the same time the subjects carried out a mental arithmetic task. The data were recorded on a store 4 tape recorder via a 16 channel data multiplexer for off-line analysis. Filter settings of 0.5–70 Hz were used.

Analysis of the electroencephalographic data was carried out off-line using a PDP-11/34 computer. From each 2.5 min recording of the EEG (eyes open and eyes closed) 16, 4 s, epochs were selected for analysis. The analogue data were low pass filtered (cut-off frequency 40 Hz) before digitisation at 128 points s⁻¹, and the mean 256 point power spectrum with 0.25 Hz resolution was computed using fast Fourier transform techniques. From the mean power spectrum the total power in each of 6 frequency bands (1.25–3.25 Hz (delta), 3.50–7.25 Hz (theta), 7.50–9.25 HZ (alpha 1), 9.50–12.25 Hz (alpha 2), 12.50–17.25 Hz (beta 1) and 17.50–32.00 Hz (beta 2)) was found. The bands were analysed for each channel (P3-01 and CZ-MM) with eyes open and eyes closed.

Auditory Evoked Responses. These were recorded between Cz and paired mastoids in response to a series of regular tones which included an occasional loud tone presented through headphones. The subjects were required to count the loud infrequent tones and ignore the frequent soft tones. They fixated on a small dot directly ahead, and vertical eye movements and the electromyogram were monitored. Evoked potentials modified by eye movement were ignored. The task lasted between 15 and 20 min and there were 40, randomly spaced, loud tones. The responses to 32 loud tones were selected to give an averaged response, and the 32 preceding responses to soft tones were used for comparison.

The recordings of the auditory evoked responses were played into a PDP-12 computer via a 16-channel multiplexer. Responses with eye movement artifacts were rejected, and out of the 40 responses a maximum of 32 were accepted. These were averaged for the soft and loud responses. Each record had 160 points, sampled at 128 Hz, and the first 32 points preceded the stimulus.

Analysis

Analysis of variance (ANOVA) was used as the data were the result of a designed experiment. Three fac-