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ELECTROENCEPHALOGRAPHIC CHANGES IN THE LATERAL SEPTUM COMPLEX FOLLOWING SYSTEMIC ADMINISTRATION OF DES-TYR^1 -α-ENDORPHIN, DES-TYR^1-γ-ENDORPHIN AND HALOPERIDOL IN RATS

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Summary

The influence of systemically administered Des-Tyr^1-α-endorphin (DTαE), Des-Tyr^1-γ-endorphin (DTγE) and haloperidol on electroencephalographic (EEG) activity of the lateral septum complex (LSC) and the frontal cortex was studied in male rats. DTαE (2 μg) significantly increased whereas DTγE (10 μg) significantly decreased the amounts of activity in the 5 Hz band. In addition, DTαE promoted production of 15 - 20 Hz activity, while DTγE decreased the amount of 20 - 30 Hz activity. EEG activity exhibited a marked variability which persisted throughout the recording session following administration of the peptides. Haloperidol markedly decreased the proportion of 10 - 15 Hz activity. The alterations in EEG of the frontal cortex were similar to those in LSC but less pronounced. The differences in the time course and frequency bands affected suggest that the effects of peptides and haloperidol on EEG activity of LSC are not mediated by the same mechanisms.

Two closely related peptides, Des-Tyr^1-α-endorphin (DTαE) and Des-Tyr^1-γ-endorphin (DTγE), both derived from the β-lipotropin hormone (β-LPH) molecule exhibit a distinctly different effect on avoidance behavior in rats. The sesequence of DTαE (β-LPH 62-76) delays extinction of active avoidance and facilitates retention of passive avoidance behaviors whereas DTγE (β-LPH 62-77) accelerates extinction of active and attenuates retention of passive avoidance behaviors (1,2). Des-Tyr^1-endorphins were detected in rat pituitary (3) and brain (4). DTαE and DTγE can be preferentially formed from the precursor molecule β-endorphin (βE; β-LPH 61-91) by exposure of the molecule to enzymes associated with enriched synaptosomal plasma membrane fraction from the rat brain (5). These results suggested that Des-Tyr^1-endorphins are endogenous peptides which may be formed from the same parent molecule and which presumably have different function in the brain.

The limbic structures and the lateral septal complex (LSC) in particular are rich and the frontal cortex (FC) is poor on the immunoreactive βE (4,6,7), the presumed source of DTαE and DTγE. In the present study, we report that DTαE and DTγE altered preferentially the electro-encephalographic (EEG) activity of LSC and that DTγE whose activity in the avoidance procedures, various "grip tests" and in self-stimulation resembled that of haloperidol (2,8,9), induced different kinds of EEG patterns in LSC than the neuroleptic.

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Materials and Methods

Experiments were carried out on 6 male Wistar rats which were equipped with monopolar electrodes (stainless-steel wires, 100 μ in diameter), implanted according to stereotaxic coordinates (10) A= 8.5 mm; L= 0.7 mm; H= 4.00 mm in the lateral septal complex and epidurally above the frontal cortex (coordinates A= 2.00 mm rostral from the bregma; L= 2.50 mm from the sagital suture). The EEG activity was recorded on the polygraphic paper and magnetic tape through a cable assembly (11). EEG was quantified according to the following procedure. Fifteen 2 sec epochs of filtered (high frequency cut off at 100 Hz) EEG were digitized (sampling rate of 250 Hz) every 2 min, and power spectra of each epoch computed and stored. From thus obtained 150 spectra, the average calibrated power spectrum per 20 min long recording period was computed. The 0.5 Hz band width in these spectra was adjusted to Hz by taking the arithmetic mean of 10 adjacent frequencies, neglecting the DC component.

Effect of drugs on spontaneous human EEG is often studies in a group of individuals which receive placebo followed on the same or different day by a single dose of the tested compound. Each individual serves as his own control and the spectra recorded after the placebo and drug administrations are compared in the paired "t" test (12,13,14). Applicability of a similar procedure to the spontaneous rat EEG was examined in two experiments in which only placebo (0.9 % NaCl solution) was given. Each of these experiments consisted of two consecutive days and the experiments were separated by an interval of 14 days. Experiments started at 11.00 o'clock by recording 30 min of control activity prior to injection. At 11.30 all animals received placebo injection and 20 min long segments of EEG activity were recorded at 12.00, 12.30, 13.30 and 15 h. Paired "t" test comparison of average spectra derived from activity of the corresponding recording periods from two consecutive days resulted in 9 - 15% significances at $p < 0.05$ and 3 - 6% significances at $p < 0.01$ indicating a treatment-independent variability in EEG activity which may lead to an erroneous evaluation of the drug effects. The variability could be attenuated by introducing quotients described by Haller (15). The quotients express differences between EEG activity in the pretreatment (control) period and periods which follow the treatment and are computed for each rat according to the following formula: $Q_{d1px} = (Z_{d1p1x} - Z_{d1p0x})$; where Z = mean power; d = day (1 = placebo, 2= treatment); p = recording period (p0 = control period prior to any treatment; p1-4= periods following treatment); x = frequency band. The paired "t" test comparison of thus computed quotients of activity in corresponding recording periods on two consecutive placebo treated days resulted in no (see example in Fig. 1A) or less than 5% significances at $p < 0.05$ level. From this it was assumed that incidence of significances at $p < 0.01$ or number of significances greater than 5% at $p < 0.05$ in comparison of quotients from the corresponding recording periods in two consecutive days in which the placebo was given on day 1 and the tested compound on day 2, is most likely to be the result of the treatment.

In each of the subsequent experiments, only one dose of a compound was studied in all 6 animals. Animals served as their own control and received saline on day 1 and tested compound (DTαE or DTγE freshly dissolved in 0.5 ml of saline, or haloperidol dissolved in 0.001 N tartaric acid and adjusted to 0.5 ml volume with saline) on day 2. The time of injections and the recording procedure was identical to that of the saline experiments. Single injection of DTαE, DTγE or haloperidol never affected avoidance behavior of the rats for more than 48 h (1,2). Thus, the minimal interval of 7 days between experiments was considered sufficiently long for normalization of the brain functions after each treatment. Effects of treatment were evaluated by comparing the corresponding quotients from day 1 and 2 in two-tailed "t" test for paired values. Thus obtained t-values were plotted or presented with S.E.M. in form of a table. The positive

values in the plots indicate an increase, the negative ones a decrease in intensity which occurred in the examined frequency band as the result of treatment. Placement of the electrodes was reconstructed from 100 μ frontal frozen sections coloured with .1% thionine.

Results

Treatment with 2 μg DTαE resulted in a significant ($p < 0.025$) increase of activity in 5 Hz band (not shown). Effect of 10 μg DTαE on LSC activity was rather complex (Fig. 1B). In the first period (30 - 50 min after the injection) there was a slight enhancement of 45 - 105 Hz activity which was in the second period followed by a significant ($p < 0.025$) increase in the amount of 10 Hz and a tendency to an increased amount of 15 - 20 Hz activity. In period 4, an increased intensity in 30 and 45 Hz band and a significant decrease in 80 Hz band was found. Treatment with 2 μg DTγE had little effect on EEG activity. Alterations in EEG following administration of 10 μg DTγE were most pronounced in the second recording period and consisted of a highly significant ($p < 0.01$) decrease in the amount of 5 Hz activity (Fig. 2A and Table I). In addition to this decrease there was also a slight decrease in 20 - 30 Hz frequency bands in periods 2 and 3. The activity in the last recording period resembled the activity in the first period. In both these periods the peptide promoted the production of frequencies above 45 Hz.

Haloperidol in a dose of 50 μg (150 μg/kg) exhibited the strongest action on EEG of LSC. The highly significant negative "t" values in 10 - 15 Hz bands of the first recording period indicated that the butyrophenone derivative markedly decreased production of activity in these bands (see Fig. 2B). A general increase in frequencies above 45 Hz which was found in this period was, with exception of 55 Hz band, below the significancy level. The marked decrease in 10 - 15 Hz activity in the first period was replaced in the second period by an increment in the 20 Hz band. In the periods 3 and 4 the "t" values were randomly distributed around zero and indicated that the effect of haloperidol had disappeared.

The "t" profiles computed for the frontal cortex activity (not shown) followed those obtained for LSC activity. However, the "t" values were smaller and reached the significancy ($p < 0.05$) only in 5 Hz band following 10 μg DTγE injection and in 10 Hz band ($p < 0.01$) after treatment with haloperidol.

Discussion

Evidence is presented that DTαE and DTγE which exhibit opposite effects on active and passive avoidance behaviors (1,2) alter EEG activity of LSC and frontal cortex in a different manner. The most pronounced differences were found in amounts of 5 - 10 Hz activity. The dose of 10 μg DTγE decreased the activity in 5 Hz band whereas 2 μg of DTαE increased it. Alterations in these bands were significant at $p < 0.010$ or 0.025. Comparison of saline/saline experiments never resulted in differences at these p levels and thus they are most likely the result of the peptide treatment. Furthermore, DTγE tended to decrease the amounts of 20 - 30 Hz frequencies. The higher dose (10 μg) of DTαE tended to increase 15 - 20 Hz activity, whereas the smaller dose of this peptide had no effect on this activity. The effects of DTαE and DTγE on activity in other frequency bands were rather complex and could be best described as an increase in variability which was particularly marked in the frequency bands above 45 Hz. This increase in variability occurred shortly after injection of the peptides and persisted throughout the recording session. The muscular activity may contaminate the high (30 Hz and higher) frequency activity in the monopolar recordings (15). There is therefore a possibility that some of the treatment-related alterations in the high (above 30 Hz) frequency

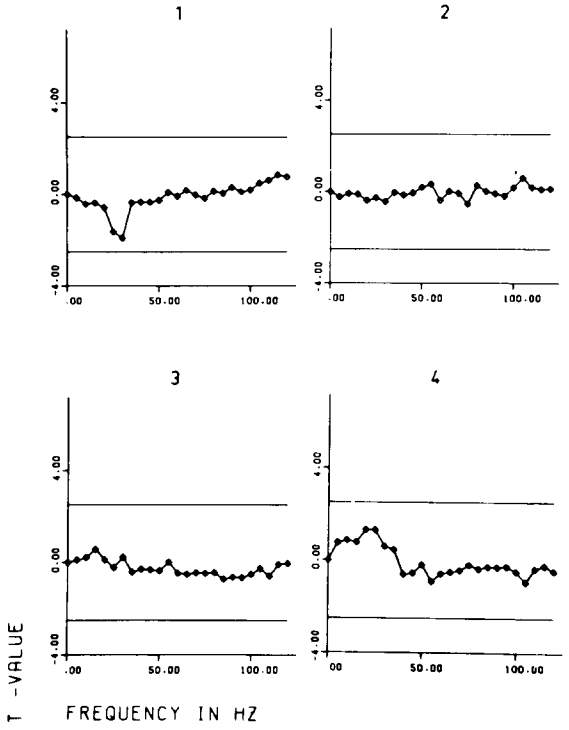

FIG. 1A

Plot of "t" values for quotients of 4 recording periods of LSC activity in two subsequent days treated with placebo. In this and other figures, the numbers above the plots indicate the recording period (explanation in the text); the horizontal lines indicate the significance level ($p < 0.05$), distance between the symbols is 5 Hz.

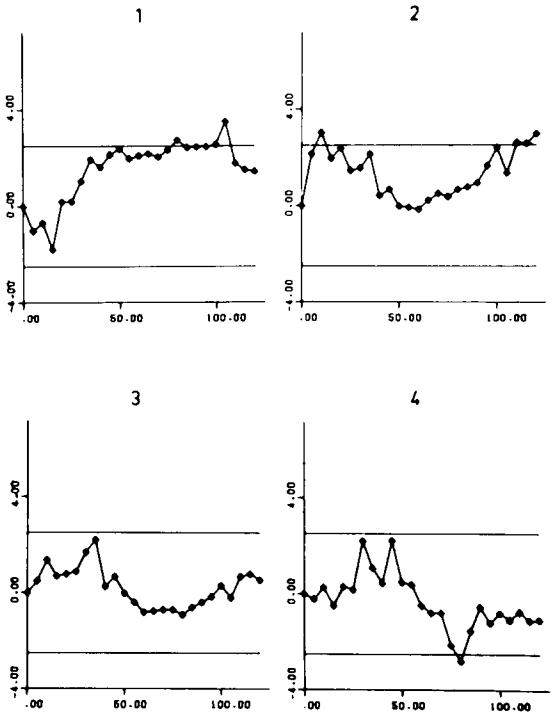

FIG. 1B

Plot of "t" values computed for quotients of 4 periods of the LSC activity in placebo and 10 μg DTαE treated days.

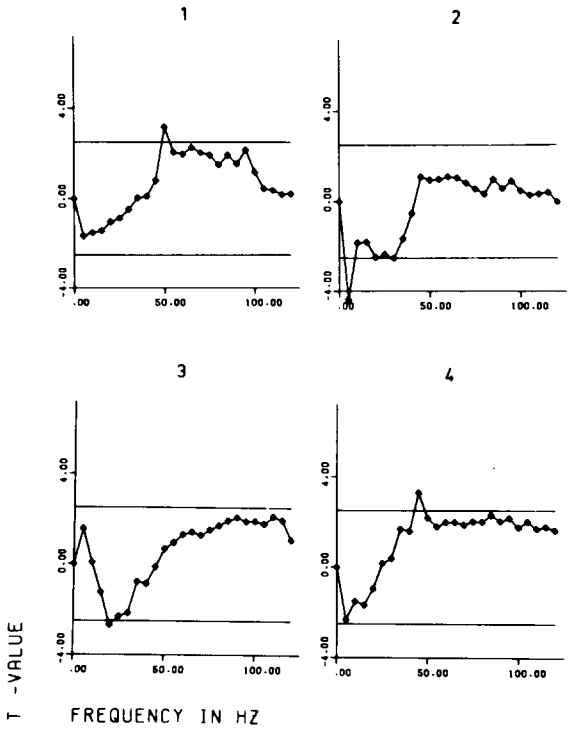

FIG. 2A

Plot of "t" values computed for quotients of 4 recording periods of the LSC activity in placebo and 10 µg DTγE treated days.

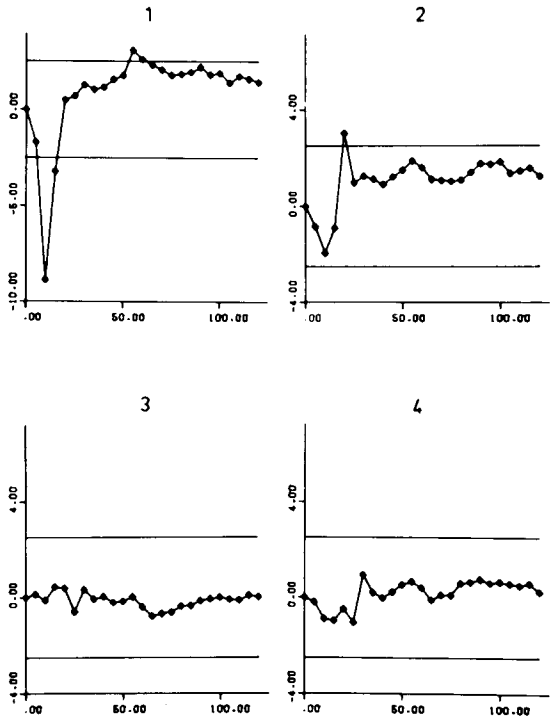

FIG. 2B

Plot of "t" values computed for the quotients of 4 recording periods of the LSC activity in placebo and 50 µg haloperidol treated days.

TABLE I

Comparison of 5 - 35 Hz Activity in the Second Period of the Saline and 10 μg DTγE Treated Days

Hz	Qd2p2 - Qd1p2	± S.E.M.	"t" (5)
5.0 Hz	- 1,408.6	312.6	- 4.380
10.0 Hz	- 755.3	412.3	- 1.832
15.0 Hz	- 694.3	386.4	- 1.797
20.0 Hz	- 296.2	113.5	- 2.610
25.0 Hz	- 207.6	83.3	- 2.493
30.0 Hz	- 215.8	79.9	- 2.702
35.0 Hz	- 101.8	61.7	- 1.651

Qd2p2 - Qd1p2 = mean differences (x 10^3) between quotients from the DTγE (Qd2p2) and saline (Qd1p2) treated days (period 2).
S.E.M.: standard error of the mean.
"t" (5): "t" value (degree of freedom).

bands near or at p= 0.05 might not entirely be due to changes in the brain activity. No epileptiform high voltage slow waves were observed in EEG records following subcutaneous administration of DTγE and DTαE. Occurrence of this type of activity in various neocortical and limbic structures has been reported following the intracerebroventricular or the intracerebral microinjections of peptides with opiate-like activity (17-26). Effect of haloperidol on EEG activity of LSC differed from that of peptides in the frequency bands affected, the magnitude and the time course. Thus, the major effect of the butyrophenone derivative consisted of a decrease in amounts of 10 - 15 Hz frequencies whereas the peptides affected the frequency bands of 5 - 10 Hz. Inferred from the "t" values, the magnitude of changes induced by haloperidol markedly exceeded the magnitude of any change induced by the peptides. Following injection of peptides, EEG remained altered throughout the recording session, whereas the changes induced by haloperidol decayed very rapidly. As shown in Fig. 2B, the frequency pattern of the activity recorded 120 min after the haloperidol injection did not differ from that recorded following the placebo injection. These dissimilarities suggest that the effects of peptides and of haloperidol are not mediated by the same mechanisms.

It cannot be excluded that the EEG effects of α- and γ-type endorphins are not the consequence of some peripheral action of the peptides, However, other experiments suggest that sufficient quantities of peptides may have penetrated the blood brain barrier and have directly affected the brain. Thus, as much as 40 pg of intact peptide was recovered from the brain of rats 15 min following intravenous injection of 1 μg ^{3}H-DTγE (Witter, 1981, personal communication) and 6 - 20 pg DTγE administered into the nucleus accumbens attenuated passive avoidance behavior in these animals (Kovács, 1981, personal communication). The monopolar derivation used in the experiment is less suited to indicate the locus of action of a systemically administered drug because it can not distinguish with certainty between the effects which occur locally under the recording electrode and the effects which originate at some distance from the recording place. However, the peptides and haloperidol affected more strongly the activity of LSC than the activity of the frontal cortex. This suggests that the compounds may have acted upon the septum itself or upon structures whose input to the septum is stronger than to the frontal cortex. Whether the different EEG effects of DTαE and DTγE may be explained by a different action of the two peptides upon the same population of neurones is currently studied. The results are compatible with the notion that DTαE and DTγE or other peptides derived from the one parent molecule may have a different function in the brain.

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