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Influence of β-Lipotropin Fragments on Responsiveness of Rats to Electric Footshock

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MENS, W. B. J. AND J. M. VAN REE. *Influence of β-lipotropin fragments on responsiveness of rats to electric footshock.* PHARMAC. BIOCHEM. BEHAV. 15(1) 27–32, 1981.—Subcutaneous administration of graded doses of neuropeptides related to lipotropin (βLPH) changed responsiveness of rats to electric footshock. γ-Endorphin and related peptides increased the susceptibility of rats, whereas β-endorphin induced a reversed effect. The enhanced responsiveness induced by DTγE, persisted for more than 24 hr and appeared to be of a central origin. Structure-activity relationship studies revealed that the sequence LPH_{65-69} may contain the active core in this respect. An increased sensitivity to electric shock within one test procedure was observed with DTγE and α-endorphin. Prior experience with the test procedure diminished or abolished this effect of α-endorphin. It is suggested that β-endorphin and its fragments play a physiological role in adaptive behavioral changes induced by peripheral stimuli.

βLPH fragments Endorphins Structure-activity relationships Electric footshock

PEPTIDES related to the C terminus of the pituitary hormone β-lipotropin (βLPH) possess morphinomimetic properties [1, 14, 18]. In addition, these peptides induce behavioral changes which seem to take place independent of opiate receptor sites in the brain. Thus, α-endorphin (βLPH_{61-76}), β-endorphin (βLPH_{61-91}) and related peptides delay [7], whereas γ-endorphin (βLPH_{61-77}) and related peptides facilitate extinction of pole jumping avoidance behavior [8]. Further studies revealed that α-endorphin has behavioral activities which in some aspects resemble those of the psychostimulant drug amphetamine and that γ-endorphin and particularly [des-tyrosine]-γ-endorphin (DTγE, βLPH_{62-77}) induce effects which in some aspects are comparable to those of the neuroleptic drug haloperidol [6, 8, 20]. These investigations include active and passive avoidance behavior, which is acquired by using escapable and inescapable electric footshock, respectively. Therefore, it was deemed of interest to study the effectiveness of various fragments of βLPH upon response of rats evoked by an electric footshock. Especially, the action of α-endorphin and of DTγE was investigated in detail. For comparison reasons, the influence of amphetamine, haloperidol and morphine on this behavior was examined.

METHOD

Animals

Male rats of a Wistar strain weighing between 140 and 160 g were supplied by Central Breeding Laboratories TNO, Zeist, The Netherlands. The animals were maintained in a temperature controlled environment (22±1°C) with a regular day-night cycle. Standard rat food and water were available ad lib.

Surgery

For intracerebroventricular application of drugs polyethylene cannulae were implanted under Hypnorm® anesthesia into one of the lateral cerebral ventricles of rats (1.5 mm lateral to the midline, 0.5 mm caudal to bregma and 4.0 mm ventral from the surface of the skull). Animals thus operated were housed in single cages. Testing of the animals was performed at four days after surgery. At the end of the experiments the animals were killed by decapitation and the position of the tip of the cannula was marked with Evans blue. After removal and incision of the brain, the position of the cannula was verified macroscopically.

Electric Footshock (EFS)

Response behavior to electric footshock (EFS) was studied in a perspex box with a grid floor as described previously [11]. After 1 min of adaptation, the rats were subjected to 20 shocks of various intensities. Each shock lasted 1 sec and the interval between shocks was 15 sec. Two blocks of each ten different shock intensities were presented to the rats (test-trial). The short circuit values of the subsequent intensities were in mA: first block (block I) 0.110, 0.042, 0.084, 0.150, 0.170, 0.046, 0.070, 0.125, 0.062, 0.058; second block (block II) 0.070, 0.046, 0.058, 0.150, 0.084, 0.062, 0.170, 0.042, 0.125, 0.110.

The response of the animals during the 1st sec after each shock was recorded. The following criteria were used:

(a) No response (0): no detectable response to shock.

(b) Flinch response (F): a slight movement with one of the legs or with the head.

(c) Jerk, Run, Vocalized, and Jump response (R): the jerk response was defined as a violent and sudden movement of the legs, head or whole body. The run response was a displacement more than $1^1/_2$ the rat's own body length. A jump was defined as a response in which all four legs left the grid floor at the same time. A vocalized response was a scream or a squeak.

Experimental Procedures

Experiment 1. This series of experiments was performed to establish whether neuropeptides and drugs changed response behavior of rats in the EFS test. Groups of 5–7 naive rats were treated with graded doses of neuropeptides or drugs. One animal received only one treatment. α-Endorphin (0.05, 0.5, 5.0 or 50.0 μg/animal) or (des-Tyr^1)-γ-endorphin (DTγE) (0.05, 0.5, 5.0 or 50.0 μg/animal) was injected subcutaneously (SC). Amphetamine (0.1, 0.3, 1.0, 3.0 or 10.0 mg/kg) was applied intraperitoneally (IP) and haloperidol (0.001, 0.01, 0.1 or 1.0 mg/kg) subcutaneously (SC). The rats were tested 0.5 hr after injection, except those receiving haloperidol, which were tested 1 hr after treatment. Rats injected with saline or vehicle (0.5 ml) were used as controls.

Experiment 2. To perform structure-activity relationships, a number of neuropeptides related to α-endorphin and DTγE were tested using the EFS procedure. In addition, two peptides related to ACTH and morphine were tested in order to characterize in more detail peptide and drug action as assessed with the EFS test. Groups of 6–18 naive rats were treated SC with 0.5 or 5.0 μg of met-enkephalin (βLPH$_{61-65}$), α-endorphin (βLPH$_{61-76}$), γ-endorphin (βLPH$_{61-77}$), β-endorphin (βLPH$_{61-91}$), βLPH$_{62-69}$, DTαE (βLPH$_{62-76}$), DTγE (βLPH$_{62-77}$), βLPH$_{65-77}$, βLPH$_{70-76}$, βLPH$_{70-77}$, ACTH$_{4-10}$ or ACTH$_{4-10[D\text{-}Phe^7]}$. Morphine (20.0 mg/kg IP) was given to 6 naive rats. One animal received only one treatment. Peptides and morphine were administrated 0.5 hr prior to testing. Animals injected with saline (0.5 ml) were used as controls.

Experiment 3. To explore time-effect relationships with respect to the ability of DTγE to affect the behavior of the animals in the EFS paradigm, groups of 7–19 naive rats were treated SC with 0.5 μg of DTγE. The animals were subjected to the EFS test 0.5, 1, 2, 4, 6 or 24 hr following treatment. Each rat was injected and tested only once. Groups of 7 animals treated with saline (0.5 ml) and tested at similar time intervals after injection as indicated for DTγE, were used as controls.

Experiment 4. In this experiment neuropeptides and drugs were injected directly into the brain ventricle, in order to determine whether the effectiveness of the peptides to alter responsiveness of the rats to EFS was of a central origin. Groups of 5–12 rats equipped with a plastic cannula into one of the brain lateral ventricles were injected with α-endorphin (0.5 ng), γ-endorphin (0.5 ng), DTγE (0.5 ng), amphetamine (0.6, 1.0 or 10.0 μg) or haloperidol (0.1, 1.0, 10.0 ng or 1.0 μg). Each animal received only one treatment. Rats injected with vehicle were used as controls. Adjusted Hamilton syringes were used to carefully inject 2 μl of solution into the ventricle. The animals were injected 0.5 hr prior to testing.

Experiment 5. This experiment was performed to analyse in more detail the effectiveness of α-endorphin to change response behavior within one EFS test. Rats treated SC with either 0.5 μg α-endorphin (n=8) or saline (n=7) 0.5 hr before testing was started, were subjected to 4 blocks of shock intensities (block I, II, I, and II in that order, $\overline{\text{TTTT}}$) without any time delay between the blocks. Subsequently, groups of 12–15 rats were exposed to one (block I, $\overline{\text{T}}$) two (block I and II, $\overline{\text{TT}}$) blocks of shock intensities and tested again after 2 hr or 24 hr (second test). Half an hour before the second test, which included two blocks of shock intensities (block I and II, $\overline{\text{TT}}$), the rats were injected SC either with 0.5 μg α-endorphin or 0.5 ml saline. Each animal was subjected to only one of the various experimental procedures.

Data Analysis

Each block of shock intensities yielded 10 responses and each test trial 20 responses. The occurrence of a certain response (0, F, or R see "Electric Footshock") was expressed as percentage of total responses for each individual animal. Since it appeared that the percentage of 0-response was the most valid and reproducable, this value is given in most of the experiments. For each treated group the mean and SEM were calculated. For statistical analysis two tailed Student's t-test (paired or non-paired) were used. The data of Experiment 1 were first analysed using the one-way ANOVA testprocedure. Since in Experiment 2 and 4 different control groups were run, the effectiveness of a peptide or drug was tested using the data of controls tested in the same experiment as the respective peptide or drug.

Drugs

The following peptides were used: met^5-enkephalin (βLPH$_{61-65}$), α-endorphin (βLPH$_{61-76}$), γ-endorphin (βLPH$_{61-77}$), β-endorphin (βLPH$_{61-91}$), βLPH$_{62-69}$, (des-Tyr^1)-α-endorphin (βLPH$_{62-76}$; DTαE), (des-Tyr^1)-γ-endorphin (βLPH$_{62-77}$; DTγE), βLPH$_{65-77}$, βLPH$_{70-76}$, βLPH$_{70-77}$, ACTH$_{4-10}$ and ACTH$_{4-10[D\text{-}Phe^7]}$. All peptides were obtained from Organon International B.V., The Netherlands. They were stored in dry form under controlled temperature and humidity conditions. The peptides were dissolved in saline. Morphine HCl (morphine) and Dexamphetamini Sulfas (amphetamine) were obtained from OPG, The Netherlands and haloperidol from Janssen Pharmaceutica, Belgium. Morphine and amphetamine were dissolved in saline and haloperidol in a 1.5% tartaric acid solution. The last solvent did not change the responsiveness of the rats in EFS. Solutions for intracerebroventricular injection were prepared using artificial cerebrospinal fluid [2] as solvent. Fresh solutions of peptides and drugs were made just prior to experimentation.

RESULTS

Experiment 1: Dose-Response Studies

Administration of graded doses of α-endorphin (0.05-50 μg; SC) to naive rats did not influence response behavior in the EFS test as compared to that of saline treated animals (ANOVA, $p>0.05$); (Fig. 1A). In contrast, DTγE affected the occurrence of 0-responses (ANOVA, $p<0.05$). In fact, 0.5 and 5.0 μg DTγE diminished the occurrence of 0-responses, and 5.0 μg increased that of R-responses. A higher or lower dose of this peptide failed to significantly influence responsiveness of the rats (Fig. 1B). Amphetamine

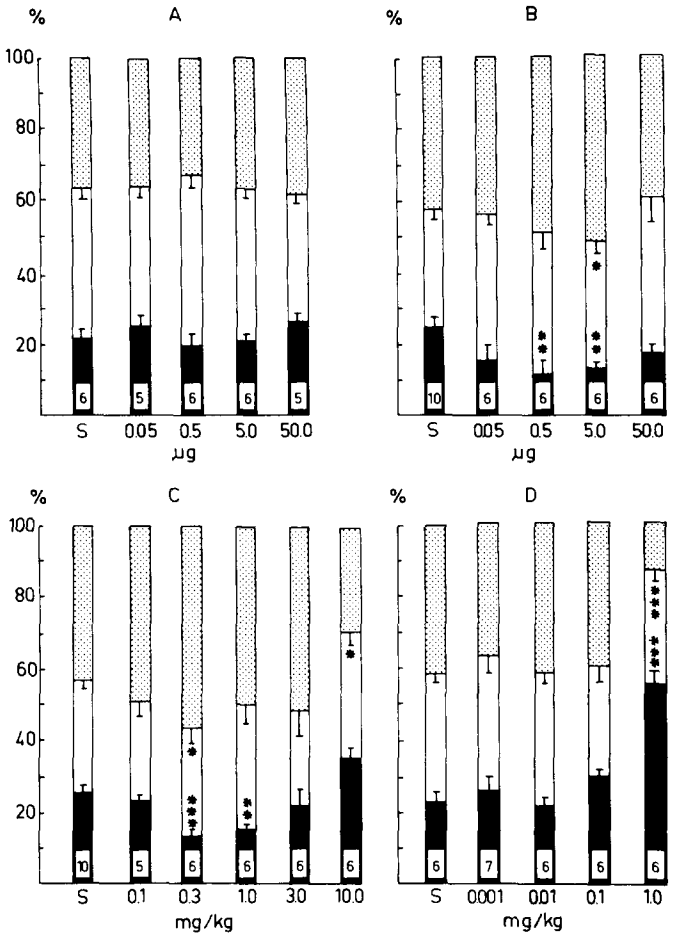

FIG. 1. Influence of graded doses of α-endorphin (A), DTγE (B), amphetamine (C) and haloperidol (D) on response behavior of rats in the EFS test as compared to saline (S) treatment. The amount of peptide or drug is plotted versus the mean percentage of no response (■), flinch-response (□) and jerk, run, jump, vocalized response (▨) as assessed in each individual animal. Vertical lines indicate SEM. The number in the bar refers to the number of animals tested. Different from saline treated animals: $*p<0.05$, $**p<0.02$, $***p<0.001$.

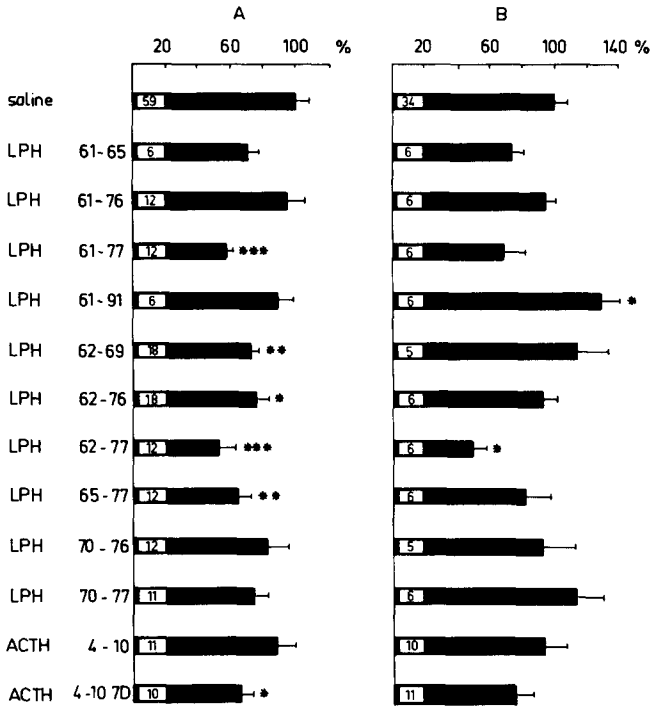

FIG. 2. Effect of various fragments of β-lipotropin in the occurrence of 0-responses in rats tested in the EFS paradigm. Groups of rats were tested 30 min after a subcutaneous injection of 0.5 μg (A) or 5.0 μg (B) of the peptides. Data are expressed as percentage of the mean value obtained in control rats simultaneously treated with saline. No differences were present between the various control groups, and therefore they are presented as one group. Horizontal bars indicate SEM. The number in the bar refers to the number of animals tested. Different from control animals treated at the same time as the respective peptide: $*p<0.05$, $**p<0.01$, $***p<0.002$.

and haloperidol appeared to influence the occurrence of both the 0- and the R-responses (ANOVA, $p<0.01$). Amphetamine (0.3 and 1.0 mg/kg; IP) reduced the occurrence of 0-responses and increased that of R-responses (Fig. 1C). A high dose of this drug (10 mg/kg) decreased the percentage of R-responses. Relatively low doses of haloperidol (up to 0.1 mg/kg; SC) did not affect responsiveness of the rats, but a higher dose (1.0 mg/kg) enhanced the occurrence of 0-responses (Fig. 1D).

In saline treated rats no changes in responsiveness to shock intensities within one EFS test was observed, in that the occurrence of 0-responses appeared to be not different between both blocks of testing (value obtained in block II was 90% ($p>0.05$) of that in block I). However, rats treated with α-endorphin or DTγE showed changes in responsiveness within one EFS test. The occurrence of 0-responses in the 2nd block was lower than that in the 1st block in rats injected with 0.5 μg α-endorphin (value obtained in block II was 55% ($p<0.002$) of that in block I), or 0.05 and 0.5 μg DTγE (respectively, 50%, $p<0.001$, and 43%, $p<0.001$). Thus, these rats appeared to be more sensitive for the delivered shocks during the 2nd block. Such effects were not observed in animals treated with the other doses of the peptides or with various doses of amphetamine or haloperidol, except in rats receiving 0.01 mg/kg haloperidol, in which the occurrence of 0-responses was lower in the 2nd block (66%, $p<0.05$).

Experiment 2: Structure-Activity Relationships

Subcutaneous injection of 0.5 μg γ-endorphin, βLPH_{62-69}, DTαE, DTγE, βLPH_{65-77} or $ACTH_{4-10[D\text{-}Phe^7]}$ elicited a reduction of the occurrence of 0-responses (Fig. 2A). However, the same dose of met-enkephalin, α-endorphin, β-endorphin, βLPH_{70-76}, βLPH_{70-77} and $ACTH_{4-10}$ did not significantly affect the behavior of the rats in the EFS paradigm. In general, a tenfold higher dose of these peptides did not influence the responsiveness of the animals as compared to that of saline treated controls (Fig. 2B). As mentioned before the occurrence of 0-responses was

TABLE 1

THE INFLUENCE OF [DES-TYROSINE¹]-γ-ENDORPHIN (DTγE) ON THE OCCURRENCE OF 0-RESPONSES IN RATS TESTED IN THE EFS PARADIGM

Time After Treatment	Saline	DTγE
0.5 hr	100 ± 8 (16)	54 ± 11 (11)†
1.0 hr	100 ± 14 (7)	59 ± 9 (9)*
2.0 hr	100 ± 18 (7)	78 ± 10 (9)
4.0 hr	100 ± 6 (7)	56 ± 7 (9)‡
6.0 hr	100 ± 17 (6)	78 ± 16 (8)
24.0 hr	100 ± 12 (19)	54 ± 8 (20)†

Groups of animals were injected subcutaneously with 0.5 μg DTγE or saline (0.5 ml) and tested on different time intervals after treatment. The data are expressed as percentage (±SEM) of the mean value obtained in the saline treated control rats.

( ) number of animals.

*$p<0.05$, †$p<0.01$, ‡$p<0.001$; different from saline treated animals.

decreased following injection of 5.0 μg of DTγE. An opposite effect was observed in rats treated with 5.0 μg β-endorphin, in which the occurrence of 0-responses was increased. A similar effect was found after intraperitoneal injection with morphine (20 mg/kg): the occurrence of 0-responses was increased to 159% ($p<0.01$).

Treatment with most of the peptides or with morphine did not change the responsiveness of the rats within one EFS test, in that the occurrence of 0-responses in the 2nd block was not significantly different from that of the 1st block of testing. Apart from α-endorphin and DTγE, only following 5.0 μg of $ACTH_{4-10}$ did the occurrence of 0-responses decrease in the 2nd block (54% of the value obtained in the 1st block, $p<0.01$).

Experiment 3: Time-Effect Relationships

The effectiveness of DTγE to change responsiveness of animals subjected to the EFS paradigm persisted for at least one day following treatment (Table 1). The occurrence of 0-responses diminished from 0.5 hr up to 24 hr after DTγE administration. At 2 and 6 hr after injection this effect did not reach the level of statistical significance due to a somewhat large variation of the obtained data. For animals that received saline no statistical significant differences between various time intervals were observed.

Experiment 4: Intracerebroventricular Application

Following intracerebroventricular administration of 0.5 ng of γ-endorphin or DTγE, the occurrence of 0-responses was significantly decreased as compared to vehicle injected animals (Fig. 3). The same dose of α-endorphin hardly changed the responsiveness of the animals. As observed after systemic injection, amphetamine (0.6 μg) increased the responsiveness of the animals to EFS, in that the occurrence of 0-responses was decreased. Higher dose levels of this drug did not significantly affect the behavior of the rats. Injection with graded doses of haloperidol, did not markedly change the behavior, although a tendency to an increased responsiveness at the low dose levels of this drug was observed.

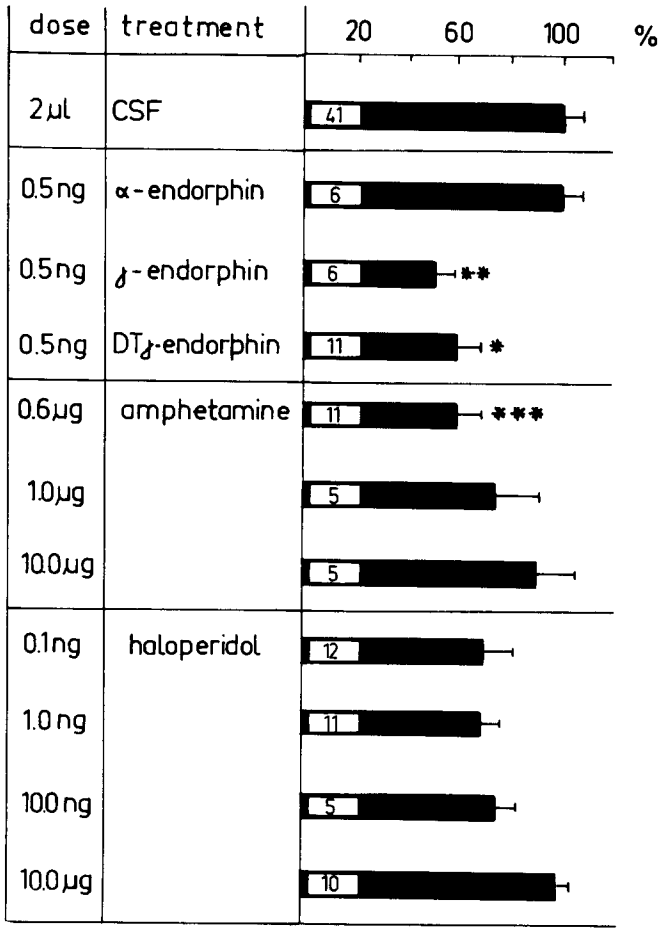

FIG. 3. The effect of intracerebroventricular administration of 5.0 ng α-endorphin, γ-endorphin or DTγE and of graded doses of amphetamine and haloperidol in rats tested in the EFS paradigm 30 min after injection. Data are expressed as percentage of the mean value obtained in control rats simultaneously treated with CSF. No differences were present between the various control groups, and therefore they are presented as one group. Horizontal bars indicate SEM. The number in the bar refers to the number of animals tested. Different from control animals treated at the same time as the respective peptide or drug: *$p<0.05$, **$p<0.02$, ***$p<0.01$.

Experiment 5: Prior Experience with the Test Procedure

α-Endorphin changed the responsiveness of the animals within one EFS test, in that the occurrence of 0-responses in the 2nd block was lower than that in the 1st block of testing (Fig. 4). Subjecting animals to consecutive 4 blocks of shock intensities, it appeared that in saline pretreated rats the occurrence of 0-responses in the 4th block was diminished as compared to that in the 1st block. This effect was enhanced in animals which were pretreated with α-endorphin. Experience with the test procedure influenced the effectiveness of α-endorphin in changing the responsiveness of the animals within one EFS test. One block of shock intensities delivered to the animals 2 hr before testing, did not change the effect of α-endorphin. However, when the animals were subjected to

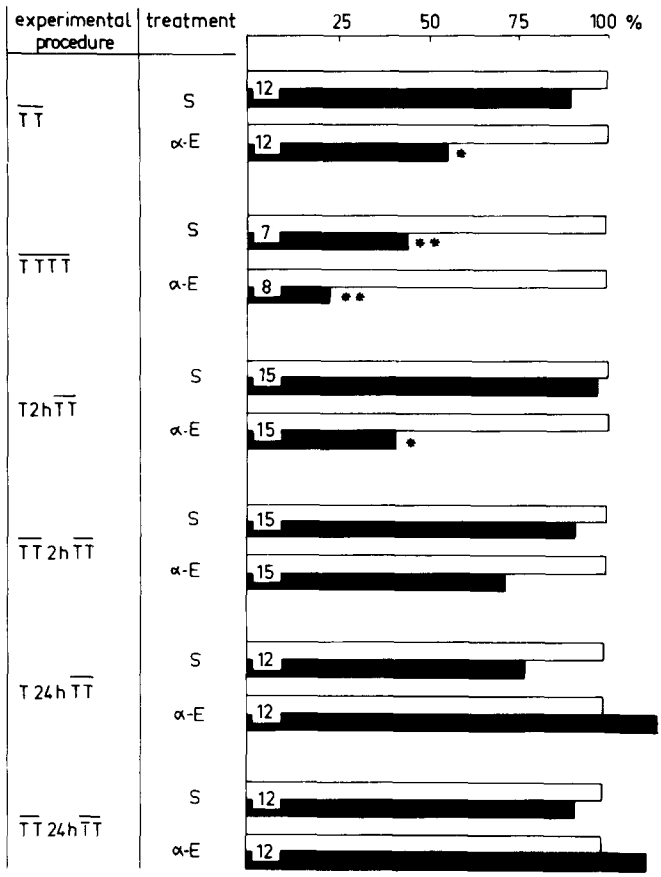

FIG. 4. The influence of prior experience with the EFS test procedure on the effectiveness of α-endorphin to change the occurrence of 0-responses in rats within one EFS test. Half an hour after subcutaneous treatment with 0.5 μg α-endorphin (αE) or saline (S, 0.5 ml) groups of animals were subjected to 2 or 4 blocks of shock intensities ($\overline{TT}$ resp. $\overline{TTTT}$). Filled bars depict the mean value obtained in the second block (or fourth block in the case of $\overline{TTTT}$) expressed as percentage of the mean value obtained in the first block (open bars). Groups of animals were subjected to one ($\overline{T}$) or two ($\overline{TT}$) blocks of shock intensities 2 or 24 hr before testing. The number in each bar refers to the number of animals tested. Different from the first block: $^{*}p<0.002$, $^{**}p<0.001$, student paired t-test.

two blocks of shock intensities 2 hr prior to testing, the effect of α-endorphin was reduced. Experience with one or two blocks of shock intensities 24 hr before testing, completely abolished the effect of α-endorphin.

DISCUSSION

The present data show that neuropeptides related to βLPH increase the sensitivity of rats to electric footshock. This effect was observed following treatment with γ-endorphin and related peptides. This increased sensitivity seems in contrast with the opiate-like activity of γ-endorphin, since morphine and related drugs have been reported to decrease the sensitivity of rats in the EFS test procedure [12]. Indeed, we found that both morphine and β-endorphin increased the occurrence of the 0-responses, which may be related to the antinociceptive activity of these entities [10, 19, 21]. Thus, the influence of γ-endorphin on the responsiveness of rats to electric footshock may take place independently of opiate receptor sites. This is supported by the more pronounced effect of the non-opiate peptide DTγE in this respect as compared to γ-endorphin.

Structure-activity studies revealed that the sequence 65–69 may contain the active core for the increased sensitivity induced by γ-endorphin related peptides. Both βLPH_{62-69} and βLPH_{65-77} appeared to be active, while met-enkephalin (βLPH_{61-65}) and βLPH_{70-77} did not induce a significant effect in this respect. However, sites outside this active core may contribute to the observed effect, because fragments containing the amino acid 77 (leucine) were more active than comparable fragments without leucine (γ-endorphin and DTγE resp. α-endorphin and DTαE). Moreover, although met-enkephalin has only a slight effect in the present test procedure, other studies suggest that low doses of this peptide can lower the pain threshold of animals [17], whereas higher dose levels induced antinociception [13]. The site of action of γ-endorphin related peptides in changing the susceptibility of rats to electric footshock may be in the central nervous system. The effects were elicited by microgram quantities when the peptides were administered systemically and by nanogram quantities following intracerebroventricular injections. The effect of DTγE lasted for at least 24 hr, indicating that the effect is of a long term nature. A similar long term action of this neuropeptide has been reported using the electrical self stimulation of the ventral tegmental-medial substantia nigra area as testprocedure [9].

It has been reported that the βLPH fragments, DTγE and α-endorphin exert behavioral activities which in some aspects are comparable to those induced by haloperidol and amphetamine respectively [8, 16, 20]. Doses of haloperidol which affect a variety of behavioral responses did not change the rat's responsiveness to electric footshock. A high dose of this drug decreased the sensitivity of the rats, presumably due to the reduced mobility. Amphetamine at rather low dose levels increased the sensitivity of the animals. Accordingly, using a modified hot plate test, Knoll [15] showed that rats treated with low doses of amphetamine were more perceptive to an unconditioned stimulus. Thus, the observed increased sensitivity induced by β-endorphin fragments may not be related to the putative neuroleptic-like effects of γ-type endorphins, but more to the amphetamine-like activity of α-type endorphins. This may be supported by the finding that the active core for the increased sensitivity may be in the sequence 65–69, while for neuroleptic-like action the amino acid leucine [77] is essential.

It is very unlikely that the effect observed in the present studies contributes to the effectiveness of βLPH fragments to change active and passive avoidance behavior. Extinction of pole jumping behavior is delayed by β-endorphin, α-type endorphins and $ACTH_{4-10}$ and facilitated by γ-type endorphins and $ACTH_{4-10[D\text{-}Phe^7]}$ [5, 7, 8]. Passive avoidance behavior is facilitated by α-type endorphins, $ACTH_{4-10}$ and $ACTH_{4-10[D\text{-}Phe^7]}$ and attenuated by γ-type endorphins [4, 7, 8]. Thus, no clear relationship exists between the influence of βLPH fragments on the sensitivity to electric foot shock and their action on avoidance behavior.

The mode of action of βLPH fragments to change the susceptibility of rats to electric footshock is unclear. Their action may be quite complex. β-Endorphin decreased this susceptibility, whereas shorter fragments induced an in-

creased sensitivity. The effectiveness of α-endorphin to change sensitivity in one EFS test seems to depend on prior experience of the rats with the test-procedure. It has been suggested that endorphins are implicated in motivational processes concerned in response to painful stimuli [7,18]. If so, these processes are affected by β-endorphin and by its fragments in an opposite way. The facts that β-endorphin fragments may be present in the brain [22], that they can be formed from β-endorphin by brain synaptosomal plasma membrane preparations [3] and that their influence on the susceptibility to electric footshock is obtained at rather low dose levels, suggest that β-endorphin and its fragments play a physiological role in adaptive behavioral changes by peripheral stimuli. In particular, the balance between the different endorphins and their fragments in the brain may be of significance in this respect, as also holds for other adaptive brain mechanisms [6].

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