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BRIEF COMMUNICATION

Effect of Org2766, an ACTH(4–9) Analogue, on Recovery After Bilateral Transection of the Fimbria Fornix in the Rat

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PITSIKAS, N., B. M. SPRUIJT, M. JOSEPHY, S. ALGERI AND W. H. GISPEN. *Effect of Org2766, an ACTH(4–9) analogue, on recovery after bilateral transection of the fimbria fornix in the rat.* PHARMACOL BIOCHEM BEHAV **38**(4) 931–934, 1991.— Facilitation of recovery after peripheral or central damage of the nervous system induced by neuropeptides was recently extensively reported. In a previous study we reported that Org2766, an ACTH(4–9) analogue, enhances recovery as assessed in a spatial orientation task after unilateral transection of the fimbria fornix in the rat. It was suggested that cross-lateral compensation by the intact fimbria fornix hippocampal system could account for the peptide-induced recovery. Therefore, the facilitatory effect of this neuropeptide was investigated in the present study after bilateral transection of the fimbria fornix. The present results indicate that Org2766 also attenuated the behavioral deficit of bilaterally transected animals in a spatial learning task, but does not affect behaviour of the lesioned animals in a passive avoidance test.

Org2766 Fimbria fornix Bilateral transection Recovery Spatial learning Morris water maze Passive avoidance

THAT the disconnection of the septo-hippocampal pathway produces severe memory impairments in animals is well known (5,10). A variety of studies proposed that some compounds, such as nerve growth factor or peptides, exert a neurotrophic action on the central and peripheral nervous system in the rat (2, 17, 19). In addition, the ACTH(4–9) analogue Org2766, which is devoid of steroidogenic activity (3), has been shown to exert beneficial effects on recovery after brain damage (7, 11, 21).

Age-related behavioural changes (8, 13, 14) and morphological characteristics in the hippocampus (8) can also be counteracted by chronic treatment with Org2766. Based on these effects, seen in cell tissue culture, peripheral and central nervous system, the hypothesis is forwarded that this neuropeptide may exert a trophic effect on neurons, especially in case of neuronal degeneration. In damaged peripheral neurons Org2766-accelerated outgrowth of axons has been demonstrated to account for the functional recovery of sensory and motor neurons (18). In another study (21), lesions in the n. accumbens were followed by postlesion reinnervation of the lesioned area in a period of about 4 weeks. In our previous study (15), which was designed analogous to the lesions carried out in the peripheral nervous system, fibres, i.e., fimbria fornix, rather than cell bodies were unilaterally transected. In this study Org2766-induced functional recovery was already seen 2 weeks after the lesion. Reinnervation of the hippocampus from the site of the transection in this relatively short period seems very unlikely. As was suggested in this study, other mechanisms, such as cross-lateral compensation, may explain the observed peptide-induced recovery.

The aim of the current experiments was to test the proposed hypothesis by determining whether the ACTH(4–9) analogue still

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exerts a facilitatory effect on recovery after extensive bilateral damage to the fimbria fornix. It is assumed that in bilaterally lesioned animals, cross-lateral compensatory mechanisms are not likely to take place. The Morris water maze and passive avoidance were chosen as the behavioural tasks for assessing behavioural deficits. The former test is sensitive to hippocampal damage (8), the latter does not depend on spatial orientation.

METHOD

Animals

Forty-one male Wistar rats (TNO, Zeist, NL) weighing between 220–240 g upon lesion of the fimbria fornix (FF) were used. The animals were randomly divided into 4 groups: sham-operated treated with saline (N = 10, sham-sal), sham-operated treated with Org2766 (N = 10, sham-Org2766), FF-lesioned treated with saline (N = 11, FF-sal) and FF-lesioned treated with Org2766 (N = 10, FF-Org2766). Animals were housed in groups of 4–5 in Makrolon cages at a temperature (21 ± 1°C) and light-controlled room with reversed day/night cycle (red light was switched on at 8.00 a.m. and switched off at 8.00 p.m.). Food and tap water were available ad lib. All experiments were carried out in the room where the animals were housed and took place between 10.00 a.m and 3.00 p.m. After lesioning, the animals were allowed to recover for 2 weeks. The experiments were started when the rats had a body weight similar to that of the day of the surgery.

Lesion and Treatment

Bilateral transections of both FF were performed according to the method described by Hefti et al. (5). The rats were anaesthetized with a subcutaneous injection (0.8 mg/kg body weight) of Hypnorm® (Duphar, Weesp, The Netherlands) containing flunisone (10 mg/ml) and phentanylcitrate (0.3 mg/ml). After placement in a stereotaxic apparatus, a specially designed knife was lowered into the brain at the coordinates 0.1 mm posterior from bregma and moved laterally from 1 to 5.0 mm at a depth of 6.5 mm. Corrected for the difference in body weight the location corresponds with the coordinates 2.0 mm posterior from bregma according to the atlas of Paxinos and Watson [(11); see Fig. 64 and 17 therein]. In the sham-operated animals the knife was moved laterally at a depth of 1 mm. Starting at the day of the lesion the sham-operated and the lesioned animals were treated with either saline or the ACTH(4–9) analogue Org2766 [H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH], a gift from Organon BV (Oss, The Netherlands). Seven injections (1 μg per animal/48 h) were given subcutaneously in an injection volume of 0.5 ml starting from the day of the lesion. All animals were subjected to the following sequence of behavioural tests: first the Morris maze and then passive avoidance. These tests began 16 days after the operation and 48 h after the last injection to avoid acute effects of the peptide treatment (20).

Morris Water Maze Task

The procedure used for testing spatial orientation in a Morris maze has been described extensively before (15). Escape latency, length of the swimming path and percentage of the swimming distance covered by the animal during the spatial probe test were registrated by a computerized image analysis system. Hardware consisted of an IBM AT computer combined with a PC vision frame grabber (Imaging Technology Inc., USA) and a CCD camera. Software for this application was developed in collaboration with Difa Measuring Systems BV (Breda, The Netherlands).

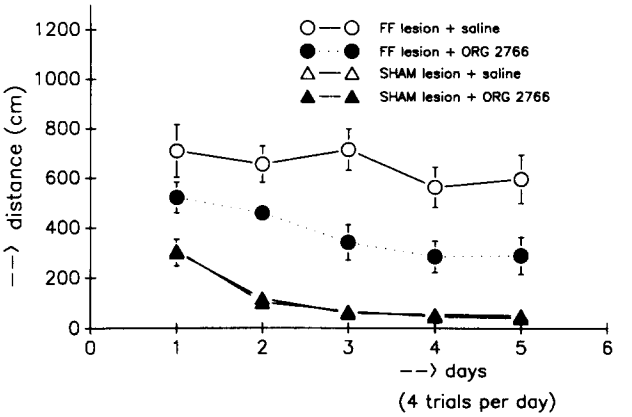

FIG. 1. For every animal the mean swum distance (cm) over the block of four trials per day was calculated. The mean distances ± SEM for the four groups are shown per day for sham-sal, sham-Org2766, FF-sal and the FF-Org2766 groups.

Passive Avoidance Test

Three days after the Morris water maze test passive avoidance behaviour was measured according to the procedure described by Ader et al. [(1); for further details see (15)].

Statistical Analysis

A two-factor analysis of variance (surgery and drug treatment) with repeated measurements was used for the comparison of the swum distance of the groups followed by an ANOVA on two factors per day and, subsequently, Tukey's HSD test. The length of the distance per quadrant was expressed as percentage of the total distance. For comparing the previously reinforced quadrant (2) with the other quadrants (1, 3 and 4), a Friedman analysis followed by a Wilcoxon test was applied. A Kruskal-Wallis test was used for evaluating the retention of passive avoidance behaviour. All statistical calculations were performed using the statistical package SYSTAT (Wilkinson, Leland, SYSTAT: The System for Statistics, Evanston, IL: SYSTAT, Inc., 1988).

RESULTS

The length of the swimming path over five days for the four different groups is plotted in Fig. 1. The analysis of variance for two factors with repeated measurements (days) showed an overall effect of the surgery on distance, $F(1,37) = 11.6$, $p<0.01$, and drug treatment, $F(1,37) = 92.5$, $p<0.01$. In addition, a significant interaction was seen between surgery and drug treatment, which is illustrated by the improvement in performance of the lesioned animals treated with Org2766, $F(1,37) = 11$, $p<0.01$.

The effect of Org2766 on the performance of lesioned animals was also tested using univariate F-tests per day. The F-values and corresponding p values for day 1, day 3, day 4, and day 5 were, $F(1,37) = 4.25$, $p<0.046$; $F(1,37) = 11.1$, $p<0.002$; $F(1,37) = 6.4$, $p<0.015$; and $F(1,37) = 5.44$, $p<0.025$, respectively; on day 2 no significant interaction was found. To assess differences between groups, the analysis of variance was followed by Tukey's HSD test. It appeared that the lesioned animals treated with Org2766 differed significantly both from the lesioned animals treated with saline, on day 1, 3, 4, and day 5: $p<0.05$, and from

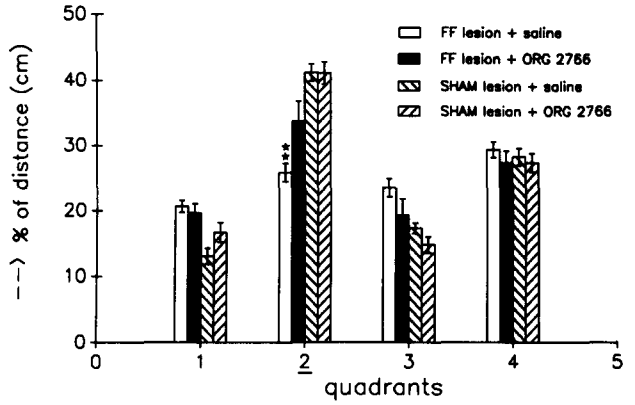

FIG. 2. The distances swum in the four quadrants upon removal of the platform from quadrant No. 2 are expressed as percentages from the total covered distance. **Indicates a significant difference between the FF-sal versus the sham-sal and sham-Org2766 groups ($p<0.01$).

the sham-operated animals on day 2, 3, 4, and day 5: $p<0.01$.

Figure 2 illustrates the results of the spatial probe task which evaluates the persistence of the animals to localize the pool zone where the platform had been placed during the acquisition trials. The sham-operated animals treated either with saline or with Org2766 swam significantly longer in quadrant No. 2, where the platform was located during the acquisition, as compared to quadrants 1, 3 and 4 (Friedman test, $p<0.01$). The FF-lesioned animals treated with saline did not swim preferentially in the pool zone where the platform was previously located (Friedman test not significant), whereas the FF-lesioned rats treated with Org2766 swam significantly more in the reinforced quadrant (No. 2) as compared to quadrant No. 1 and 3 (Friedman test, $p<0.01$), but not versus quadrant No. 4. Moreover, the percentage of the distance covered in the "correct" quadrant by the sham-operated rats was significantly higher (Kruskal-Wallis test: chi-square = 22.0, $p<0.01$) as compared to the FF-lesioned rats treated with saline, but did not differ from the performance of the FF-lesioned rats treated with Org2766.

In Fig. 3 the retention latencies in a passive avoidance test are shown. Both lesioned groups displayed shorter latencies as compared to the sham-operated groups (Kruskal-Wallis test, Chi-square = 24.6, $p<0.01$). No effect of Org2766 was revealed in this task.

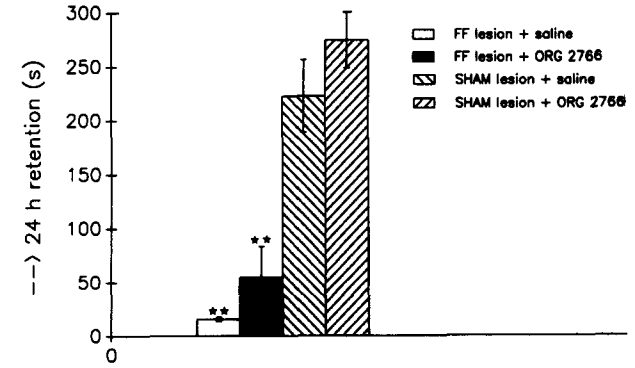

FIG. 3. The latency to reenter (s) (mean ± SEM) 24 h after the shock in a passive avoidance task. **Indicates significant differences between both sham-sal versus FF-sal and sham-Org2766 versus FF-Org2766 ($p<0.01$).

DISCUSSION

Our results, in agreement with previous studies, show that bilateral transections of FF abolish place learning (10,16). In the present study the effect of Org2766 was assessed in a bilateral transection which seems to produce a more severe deficit compared to that induced by the unilateral transection of the FF (15). The FF-lesioned rats treated with saline showed an impaired performance in a place navigation task and were unable to locate the site of the platform during the spatial probe test. All sham-operated animals acquired this task quickly. The FF-lesioned animals treated with Org2766 had markedly shortened their latencies and lengths of the swimming path throughout trials and demonstrated a preference for the previously reinforced quadrant of the platform not different from those expressed by the sham-operated animals. However, the performance of these rats was less efficient during the acquisition of this spatial task in comparison with the performance of the sham-operated animals, but was more efficient than the performance of the FF-lesioned animals treated with saline. Passive avoidance behaviour showed a good retention of the sham-operated rats and a poor performance of all the lesioned animals. Apparently, Org2766 treatment was not effective in this paradigm. Org2766 did not influence the behaviour displayed by the sham-operated animals in either of the tasks.

The present lesion, which was bilateral and produced severe damage on both sides of the hippocampus, leaves two possibilities for peptide-facilitated recovery. First, partial reafferentiation may be realised by reactive synaptogenesis in posterior connections of the hippocampus and, second, extra-hippocampal systems may be involved in compensatory actions. Since in these bilaterally lesioned animals enhanced recovery is seen, contralateral compensation is not necessarily the only mechanism which accounts for facilitated recovery in unilaterally lesioned animals. Thus the proposed hypothesis in the previous study, involving the contralateral side as a possible site of action for Org2766, is not supported by the results obtained in the present study.

Since histological inspections of the lesion did not show any reestablished connection from the site of the transection to the hippocampus 28 days after surgery, the improved recovery induced by Org2766 cannot be attributed to sprouting and reinnervation at the site of the lesions as is shown in peripheral regeneration (2) and in the paradigm of Wolterink et al. (21) for studying recovery of the central nervous system. Probably, the site of action of this neuropeptide may involve compensatory actions of other neuronal networks than impaired FF fibres. Functional recovery in the Morris maze was observed 2–3 weeks after the lesion, thus reafferentiation accomplished by long-term compensatory collateral sprouting seems unlikely (3). Whether compensatory synaptic reorganization either caused by reactive synaptogenesis or by changes in the already existing synaptic circuitry is located in posterior hippocampal connections or elsewhere remains to be established. A partial reafferentiation of posterior hippocampal connections could account for the behavioural dissociation seen during recovery. ACTH-induced functional recovery involving compensatory actions has also been suggested by Igarashi et al. (6).

In conclusion, Org2766-enhanced functional recovery has been shown to take place in various paradigms and involving neuronal outgrowth and compensation by other neuronal networks. Whether different mechanisms of action in peptide-induced regenerative processes are active simultaneously or depend on the site and the nature of the damage and the behavioural deficits involved remains to be elucidated.

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