Peptides, Vol. 11, pp. 911–914. © Pergamon Press plc, 1990. Printed in the U.S.A. 0196-9781/90 $3.00 + .00

The ACTH/MSH(4–9) Analog Org2766 Improves Retrieval of Information After a Fimbria Fornix Transection

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Received 5 March 1990

PITSIKAS, N., B. M. SPRUIJT, S. ALGERI AND W. H. GISPEN. *The ACTH/MSH(4–9) analog Org2766 improves retrieval of information after a fimbria fornix transection.* PEPTIDES **11**(5) 911–914, 1990.—The fimbria fornix of male Wistar rats was transected unilaterally after they had been successfully trained in the Morris maze and the passive avoidance task. Sham-operated and lesioned animals were treated either with Org2766 or saline for two weeks. Subsequently, the performance of all groups was tested again starting two days after the last treatment. The lesioned animals showed a deficit in performance in both tasks, indicating interference of the lesion with retrieval of information. Org2766 improved the poor performance of the lesioned animals in the Morris maze, but not in the passive avoidance task.

Org2766 Retrieval Spatial orientation Avoidance Fimbria fornix

NORMAL hippocampal functioning is essential for spatial orientation (14,15). Damage to the septo-hippocampal pathway produces cognitive deficits in tasks involved in spatial orientation (11, 12, 19). The ACTH/MSH(4–9) analog Org2766 is known for its acute effects on various behavioral paradigms (3) and for its efficacy on functional recovery of the peripheral nervous system (2, 4, 21). Beneficial effects of Org2766 on the central nervous system have been established in lesioned and aged rats: the peptide improved impaired reversal learning of rats bearing bilateral lesions in the parafascicular area (13), facilitated recovery after neurotoxic lesioning of the N. accumbens (23), and Org2766 enhanced social attention and ameliorated spatial learning in the aged rat (18).

In a previous study (19), Org2766 accelerated functional recovery of a spatial learning task after a fimbria fornix lesion. No distinction could be made between effects of the peptide on either acquisition or retrieval of spatial information. The purpose of the present study was to investigate whether unilateral fimbria fornix transection impaired the retrieval of previously acquired—prior to the lesion—spatial information and whether the ACTH/MSH(4–9) analog, Org2766, counteracts such an impairment. The behavioral changes were assessed in a Morris maze, an established spatial learning task (11) and in a passive avoidance task; the latter test is a learning task that seems less dependent on spatial orientation.

METHOD

Animals

Forty-eight male Wistar rats (TNO, Zeist, NL), weighing between 200–220 g at the time of surgery, were used. Animals were housed in groups of 4 to 5 in Makrolon cages in a light-controlled room with reversed day/night cycle at a constant temperature of 21°C. White light was switched off and red light switched on at 0800 hr and at 2000 hr red light was switched off and white light turned on. Food and water were available ad lib. All experiments took place in the room where the animals were housed (from 1000 to 1500 hr) to prevent stress induced by transportation.

Behavioral Testing

The Morris maze pool (110 cm diameter and 50 cm deep) was filled with warm water of 26°C mixed with white chalk powder. Behavioral testing took place under a reversed day/night cycle and dim red light. Briefly, on 5 consecutive days all groups received a block of four trials. Four starting points divided the pool into four quadrants of equal size. Their sequence was changed daily. If the animals did not find the platform within 120 sec, the experimenter placed them onto it for 30 sec. The platform (8 cm dia., 1 cm

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below the surface of the water) was located in a fixed position in the middle of quadrant 2, equidistant from the center and the wall of the pool. Swimming patterns were registered 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, NL).

Three days after the Morris maze test passive avoidance behavior was measured according to the procedure described by Ader *et al.* (1). Rats were subjected to 3 pretraining trials; the last trial was followed by an electric footshock. After entering the dark compartment with 4 paws, the guillotine door was closed and a shock of 0.4 mA, lasting 2 sec, was given. Retention of information was assessed by measuring the time to reenter the box (latency with a maximum of 300 sec) 24 hr after the shock.

Lesion and Treatment

Unilateral transection of the left fimbria fornix (FF) was performed using a method presented by Hefti *et al.* (6). The rats were anesthetized with a subcutaneous injection of Hypnorm (Duphar, Weesp, NL) containing flunisone (10 mg/ml) and phentanylcitrate (0.2 mg/ml). After placement in the stereotaxic apparatus, a specially designed knife (6) was lowered into the brain at 0.1 mm posterior from bregma and moved laterally from 1.0 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 [(16); see Figs. 64 and 17]. The distance between bregma and interaural line for our animals was about 8 mm. 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 Org2766 [H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH], a gift from Organon BV (Oss, NL). Seven injections, 1 μg per animal once every 48 hr, were given subcutaneously in an injection volume of 0.5 ml.

Procedure

Training of the animals in the Morris maze and in the passive avoidance test started two weeks prior to surgery. Two days after the last training session the animals were lesioned and divided randomly into the following groups: sham-lesioned treated with saline (n = 12), sham-lesioned treated with Org2766 (n = 13), FF-lesioned treated with saline (n = 12) and FF-lesioned treated with Org2766 (n = 11). During a recovery period of two weeks animals were treated as mentioned above. A second Morris maze procedure similar to the first was carried out 16 days after the lesion; after completion of the swimming task the second retention of passive avoidance behavior was assessed 30 days after the shock trial.

Statistics

Evaluation of the data of the first and the second Morris maze was performed by an analysis of variance with repeated measurements on two factors (surgery and treatment). Both latencies and distances to reach the platform have been registered. Since the distances the animals swam do not involve speed (motor abilities), this parameter has been presented here. For testing retrieval the last trial of the first test was compared with the first trial of the second test. This comparison was also done applying an ANOVA with repeated measurements on two factors (surgery and treatment). To assess differences between means of groups on each day after the lesion the ANOVA was followed by a Tukey HSD test.

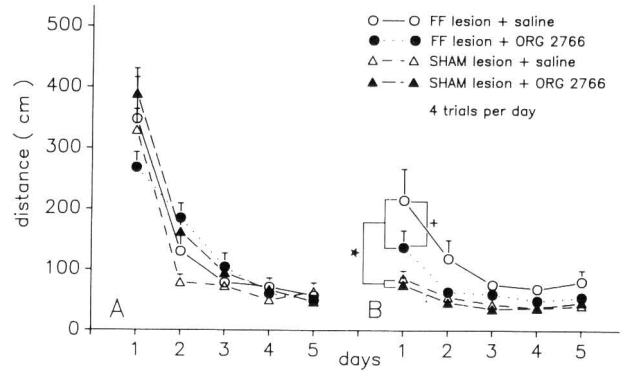

FIG. 1. Morris maze. The left panel (A) shows the mean (± SEM) distance of a block of four trials per day for the four groups of animals. No significant differences were detected. Panel B at the right side shows the performance after the lesion. The lesioned animals differed significantly from the sham-lesioned group (indicated by *) and the lesioned group, which received Org2766, differed significantly from the control lesioned animals (indicated by +); for further explanation and p values see text.

For comparing the latencies between groups obtained in the passive avoidance task a Kruskal-Wallis analysis of variance was used. Retrieval of information within the groups was tested by comparing retention before and after the lesion using a Wilcoxon test. All statistical calculations were performed using the statistical package SYSTAT (Wilkinson, Leland, SYSTAT: The System for Statistics. Evanston, IL: SYSTAT, Inc., 1988).

RESULTS

Morris Maze

Mean lengths of the swimming paths (distance) covered by the animals to reach the platform are plotted in Fig. 1. The results of the performance of the first Morris maze (before surgery) are shown in the left panel of Fig. 1. No systematic statistical differences between the four groups could be assessed over the days before the lesion. The data of the second test, after the lesion, are shown in the right panel. Overall effects showed that sham-lesioned groups differed significantly from lesioned animals: latency, $F(1,44) = 21.2$, $p<0.001$, and distance, $F(1,44) = 15.9$, $p<0.001$. The Org2766-treated animals showed a decrease in distance in the second Morris maze performance, $F(1,44) = 3.6$, $p<0.05$. As a consequence of training all groups showed an effect of trials over the days, $F(4,176) = 21.5$, $p<0.0001$. An interaction effect of trials with surgery showed that lesioned animals displayed a longer swimming distance over the days, $F(4,176) = 4.7$, $p<0.001$. For the analysis of retrieval, data obtained on the first day after lesioning were inspected more in detail. On the first test day after surgery both lesioned groups showed a significant increase in distance, $F(1,44) = 9.7$, $p<0.01$, as compared to their performance during the last trial before the surgery. An ANOVA followed by a Tukey HSD test yielded significant differences between both sham-operated groups and the FF-saline group, and a significant difference between the FF-Org2766 and the FF-saline group, $F(3,44) = 4.5$, $p<0.007$; Tukey HSD $p<0.05$. No differences between the sham-operated groups were observed.

Passive Avoidance Behavior

Figure 2 shows the retention of the animals in a passive avoidance task. All groups exhibited similar performance during the first retention trial before the lesion (Kruskal-Wallis, $p>0.05$).

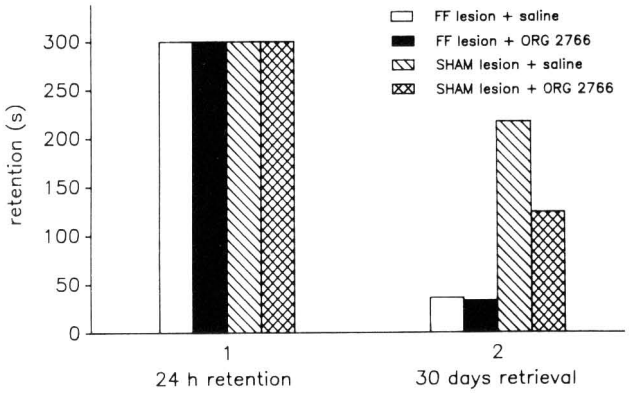

FIG. 2. Passive avoidance. Median latencies prior to the lesion and 24 hr after the shock are shown (left side) for the four groups of animals (FF-saline, FF-Org2766, SHAM-saline and SHAM-Org2766); interquartile ranges are, respectively, 0, 78, 227.5 and 241. At the right side median latencies are depicted with interquartile ranges, respectively, being: 180.5, 286, 250, 262. Lesioned animals differed significantly from sham-operated animals, $p<0.05$.

After the lesion both lesioned groups differed significantly from the sham-operated controls ($p<0.05$). Comparing the two retention trials within groups both lesioned groups performed worse than before the lesion (Wilcoxon test, two-tailed, $p<0.01$), whereas no difference was found for the sham-operated controls. The lesioned group treated with Org2766 did not improve in avoidance behavior as compared to the controls.

DISCUSSION

The results clearly demonstrate a deficit in the performance of the lesioned animals after surgery. Apparently, the lesion of the FF impairs retrieval of information. Interestingly, in the Morris maze the Org2766-treated lesioned rats performed significantly better as compared to their controls, although their performance did not reach the level of the sham-lesioned rats. A serious deficit in performance of the lesioned animals was seen in the passive avoidance task. However, Org2766 did not affect the behavior of the lesioned animals in this task. These results are in line with the outcome of another study using FF lesion prior to training, thus involving both acquisition and retrieval (19). Chronic treatment with Org2766 specifically improved the performance in the Morris maze whereas passive avoidance behavior was not affected. Thus, hippocampal functioning seems to be improved in aged (9,18) and FF-lesioned animals as assessed in spatial orientation tasks after treatment with Org2766. Disruption of the limbic system by lesioning the FF interfering with processes of attention/retrieval has also been suggested by Van Wimersma Greidanus *et al.* (22).

Beneficial effects of Org2766 on recovery following brain damage have been shown in several studies (8, 13, 23). Nonetheless, in another study it appeared that the efficiency of hippocampal-lesioned animals in a hole board task was not affected by Org2766, only an attentional deficit was attenuated by Org2766 (5). However, these lesioned animals were exceptionally successful in the two-hole board task. In the same study the impairment of neocortical-lesioned animals was not improved by Org2766 treatment. The sham-operated animals treated with Org2766 showed substantial impairments in their performance. In a study involving several posterior parietal cortical lesions an increase in errors was found in a spatial alternation task during a chronic Org2766 treatment (10). The variability in results seen after Org2766 treatment following brain injury in the different studies may be caused by several variables: the nature of the damage, the site of the lesion, the regime of treatment and the features of behavioral testing.

In peripheral neuronal recovery after nerve damage the efficacy of Org2766 on axonal sprouting is well established (2, 4, 21). Similarly, neurotrophic effects of Org2766 on neuronal outgrowth in cell tissue culture have been demonstrated (20). Long-term peptide treatment of rat embryonic cerebral cells resulted in an increase of neuronal networks and an increase of acetylcholinesterase (17). In view of the variability in effects of Org2766 on behavioral recovery after brain damage and considering the severity of fimbria fornix transection, a peptide-induced facilitation of outgrowth of the damaged neurons appears an unlikely explanation for the observed behavioral changes seen in this study after two weeks. Remodeling of other neural networks compensating for the impaired function is an alternative explanation. Igarashi (7) reported that ACTH(4–10) modified the characteristics and time courses of vestibulo-spinal and vestibulo-oculomotor balance compensation following labyrinthectomy in the squirrel monkey. The present FF lesion seems an appropriate paradigm for studies on mechanisms involved in Org2766 enhanced functional recovery after brain damage.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Marlou Josephy for her advanced biotechnical assistance and Mrs. M. Mens for her help in preparing the manuscript. This study was supported by a Constantijn and Christiaan Huygens Career Development Award to Berry Spruijt received from the Netherlands Organization for the Advancement of Pure Research (NWO).

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