A Neurotrophic Analogue of $ACTH_{4-9}$ Protects against Experimental Allergic Neuritis

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Demyelinating diseases form an important group of life-threatening neurological disorders that await effective pharmacotherapy. In these syndromes, the underlying mechanism appears to be a cell-mediated immune response directed against the myelin components.[1] Current therapeutic strategies are based on the use of antiinflammatory and immune-suppressive drugs. These drugs may render the patient more susceptible to concomitant infections and possible relapses. We speculate that the application of a neurotrophic peptide can be of significance in the treatment of these demyelinating diseases. We used a synthetic $ACTH_{4-9}$ analogue [H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH, ORG2766], a degradation-resistant peptide devoid of corticotrophic and melanotrophic activity and known to have neurotrophic properties,[2] in the experimental allergic neuritis model (EAN). EAN is an established animal model of the Guillain-Barré syndrome,[3] a human demyelinating syndrome of the peripheral nervous system. EAN was induced in female Lewis rats (160–170 g) with a subcutaneous injection of purified peripheral myelin (5 mg freeze-dried myelin/ml phosphate-buffered saline) in complete Freund's adjuvant (CFA, 4 mg M. Tuberculosum).[4] These rats were divided *ad random* into three groups. One group served as an age-matched control group (n = 10); these rats were inoculated with CFA and were treated with subcutaneous (sc) saline injections. EAN was induced in the two other groups with sc myelin-CFA injections (0.2 ml emulsion/rat) into the dorsum of the hind paws. One group received Org 2766 (75 μg/kg) sc every 48 h (n = 10), whereas the other group received saline injections (n = 10). Starting one day after immunization, the animals were submitted to a functional test to assess their motor function. Rotarod performance was analyzed according to Kaplan and Murphy[5] with minor modifications according to Gipon.[6] In the rotarod test, the motor performance of a rat is tested by measuring the ability of the rat to stay on a rotating rod. Rotarod performance was measured daily in three sessions of 2 minutes, beginning at day 0 (day of inoculation) until rats were fully recovered (day 35 pi). The investigators performing the functional analysis were unaware of the treatment a given rat had received. At the end of the experiment, the treatment codes were broken after data analysis had been completed.

Approximately two weeks after inoculation with myelin, the rats began to display clinical symptoms of EAN, which were most pronounced three weeks postinoculation (pi). Subsequently the symptoms gradually disappeared. As expected, the age-matched control group showed no symptoms at all. As illustrated in FIGURE 1, rotarod performance began to decrease drastically 14 days pi. In the

first two days after the onset of the first symptoms (day 14–day 15 pi), Org 2766–treated animals did not differ from saline-treated rats. However 18 days pi, peptide-treated animals began to recover in contrast to the control animals. These Org 2766–treated Lewis rats were fully recovered after 22 days pi. The saline-treated EAN group began to recover, at the earliest, after 21 days pi. As a result of the peptide treatment, rotarod performance was significantly improved during the entire exacerbation of EAN (median time spent on rotarod, day 20 pi: EAN-saline rats 11 s, EAN-peptide rats 46 s). The recovery periods of both the EAN-

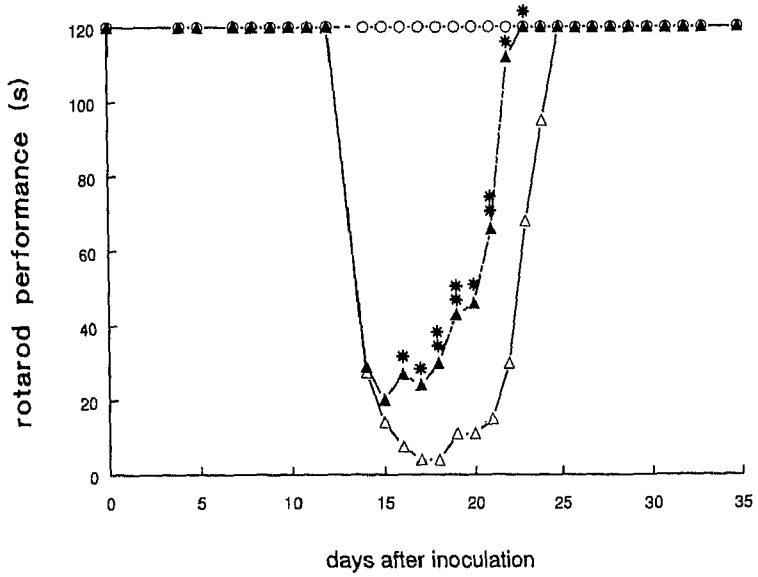

FIGURE 1. Rotarod performance test. Rotarod performance scores are presented as medians. Solid line (open triangles, $n = 10$) represents Lewis rats with EAN treated with 0.5 ml saline; dashed line (closed triangles, $n = 10$) represents Lewis rats with EAN treated with 75 μg Org 2766/kg body weight in 0.5 ml saline; dashed line with open circles represents age-matched, control rats treated with 0.5 ml saline ($n = 10$). Saline and the peptide were administered by subcutaneous injections in the neck every 48 h. Statistics: Kruskal-Wallis test followed by Mann-Whitney U-tests (EAN-Sal group vs. EAN-Org 2766 group): $^{*}p < 0.05$, $^{**}p < 0.01$.

groups treated with saline or Org 2766 were 4 days. During the last period of recovery the difference in rotarod performance between the groups neither increased nor decreased, suggesting that the peptide effect is confined to the first (five) days of impaired rotarod performance.

Thus, in this measure of motor function, i.e., performance on a rotating rod, peptide treatment provided a marked protection against EAN-related deficits in motor function. This beneficial effect underscores the potential application of this peptide in the treatment of peripheral (demyelinating) nerve disorders and may provide an alternative or a supplementary treatment to regular immune-based

therapies. The current results merit further investigations of this $ACTH_{4-9}$ analogue and its ameliorative effects in EAN or in the Guillain-Barré syndrome. Therefore, we intend to evaluate the putative neuromodulative action of the peptide in EAN with more functional tests, such as walking pattern analysis which is a more sensitive method of assessing motor function, and with histological evaluation.

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