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REGENERATION AND PROTEIN SYNTHESIS IN THE SPINAL CORD: THE INFLUENCE OF ACTH-LIKE PEPTIDES ON CELL-FREE PROTEIN SYNTHESIS

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To measure protein synthesis at the level of the cell bodies of motoneurons of the sciatic nerve, anterior horns were punched out of the spinal cord using microdissection techniques and a cell-free system was prepared. Injections of $ACTH_{1-16}$ and $ACTH_{4-10}$ were shown to enhance protein synthesis in the cell-free system, 1.5 h after treatment. Neither crush lesion of the sciatic nerve nor systemic treatment with $ACTH_{4-10}$ produced clear changes in overall protein synthesis. Thus, the increase in overall protein synthesis by acute treatment with ACTH-like peptides cannot explain the previously reported stimulation of axonal regeneration after nerve crush.

ACTH-like neuropeptides are known to influence brain and behavior in animals and man by a direct effect on the central nervous system [14]. For instance, the influences of ACTH-like peptides on protein synthesis in vivo, in vitro and in a cell-free system are well-documented, and it has been suggested that these effects are the result of a trophic action of ACTH on nerve tissue [4, 10]. Therefore it was considered possible that such peptides might facilitate the repair mechanisms which are operative after brain or nerve damage [8]. Indeed, it has recently been shown by Strand and Kung [12] and ourselves [1] that ACTH can accelerate the recovery of neuromuscular function after sciatic nerve crush and enhance outgrowth of regenerating fibers [3, 12]. Whether the stimulation of regeneration by ACTH-like neuropeptides is mediated by an influence on protein synthesis was studied in a cell-free system [10]. Previously, it has been shown that changes in protein synthesis in vivo are reflected in a cell-free system [4, 9, 10, 13].

The peptide sequence (1–16), containing all central effects but without effects on the adrenal cortex [14], or (4–10), the shortest sequence, containing the activity on

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regeneration [1], was injected 90 min before death by decapitation. The lumbar part (L2–L5) of the spinal cord (about 80 mg) was quickly dissected and rinsed with an ice-cold buffer, pH 7.6 (50 mM Tris-HCl, 25 mM KCl, 2 mM $MgCl_2$) containing 0.25 M sucrose, placed in an ice-cold metal holder (X) and cut with a slicer (Y), consisting of fixed razor-blades, 1.3 mm apart (see Fig. 1A). Using binoculars the ventral horns of the gray substance in the slices were needle-punched (1 mm internal

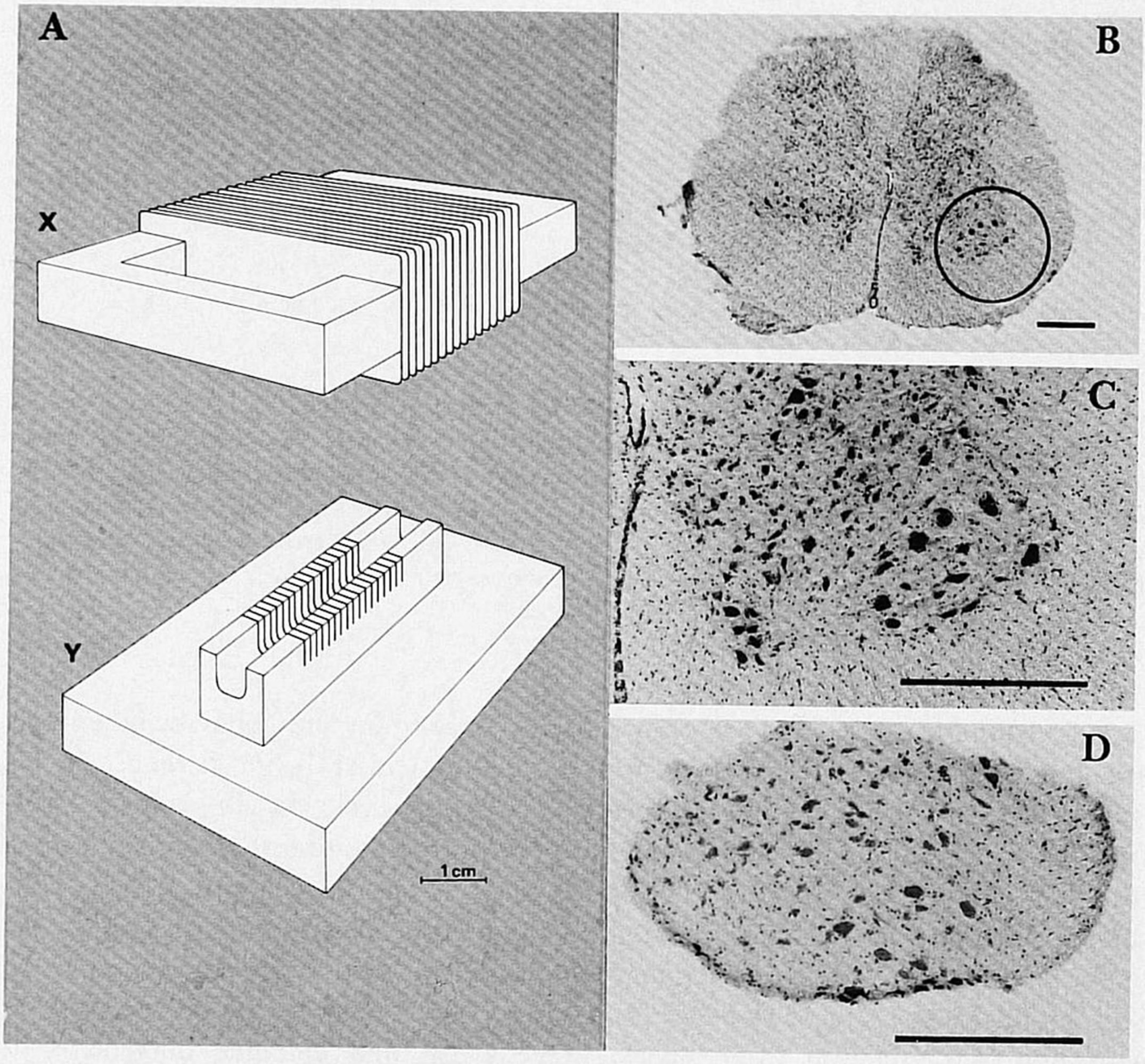

Fig. 1. A: apparatus developed for slicing rat spinal cord. The lumbar part of the spinal cord is placed in the metal holder (X) and cut into slices with the slicer (Y) consisting of fixed razor blades, 1.3 mm apart. B: hematoxylin–eosin-stained section of a slice of the lumbar part of the spinal cord. The large motoneurons concentrated in the ventral horn are clearly visualized by the basophilic dye. The site of the punch is indicated at one site of the circle. Bar = 0.5 mm. C: higher magnification of the anterior horn of the lumbar spinal cord. In addition to the darkly stained large motoneurons, the nuclei of other neurons (intermediate sized) and glial cells (small dots) are now also visible. Bar = 0.5 mm. D: punch obtained from the anterior horn of the lumbar spinal cord. This material was used to prepare a cell-free system. Bar indicates 0.5 mm.

diameter; see Fig. 1B–D). This procedure required pooling of the material of two animals in order to prepare a sufficient amount of the cell-free preparation for protein synthesis.

An aliquot equivalent to 0.140 mg of protein of the postmitochondrial fraction was incubated for up to 1 h in 0.1 ml of an incubation medium containing (final concentrations): 200 mM KCl, 12 mM $MgCl_2$, 0.5 mM ATP, 0.1 mM GTP, 6.25 mM creatine-phosphate, 0.025 mg/ml creatine kinase, 6 mM β-mercaptoethanol and 50 mM Tris-HCl, pH 7.6. Ten μCi L-[4,5-^{3}H]leucine (120–180 mCi/mmol, Amersham) was added to start the incubation. The incorporation into proteins was measured over the time intervals 0, 10, 20, 30 and 60 min. Incubations were in triplicate or 5-fold. Two-way analysis of variance was performed, using the mean of all time points derived from individual animals of the various groups of treatment. Treatment with 10 μg $ACTH_{4-10}$ (Fig. 2A, B) resulted in a 20% higher activity. A similar stimulation of 24% was observed after injection with 50 μg $ACTH_{1-16}$ (Fig. 2B). Comparable changes in protein synthesis were obtained under various in vivo and in vitro conditions after $ACTH_{4-10}$ treatment [4]. In the present in vivo/in

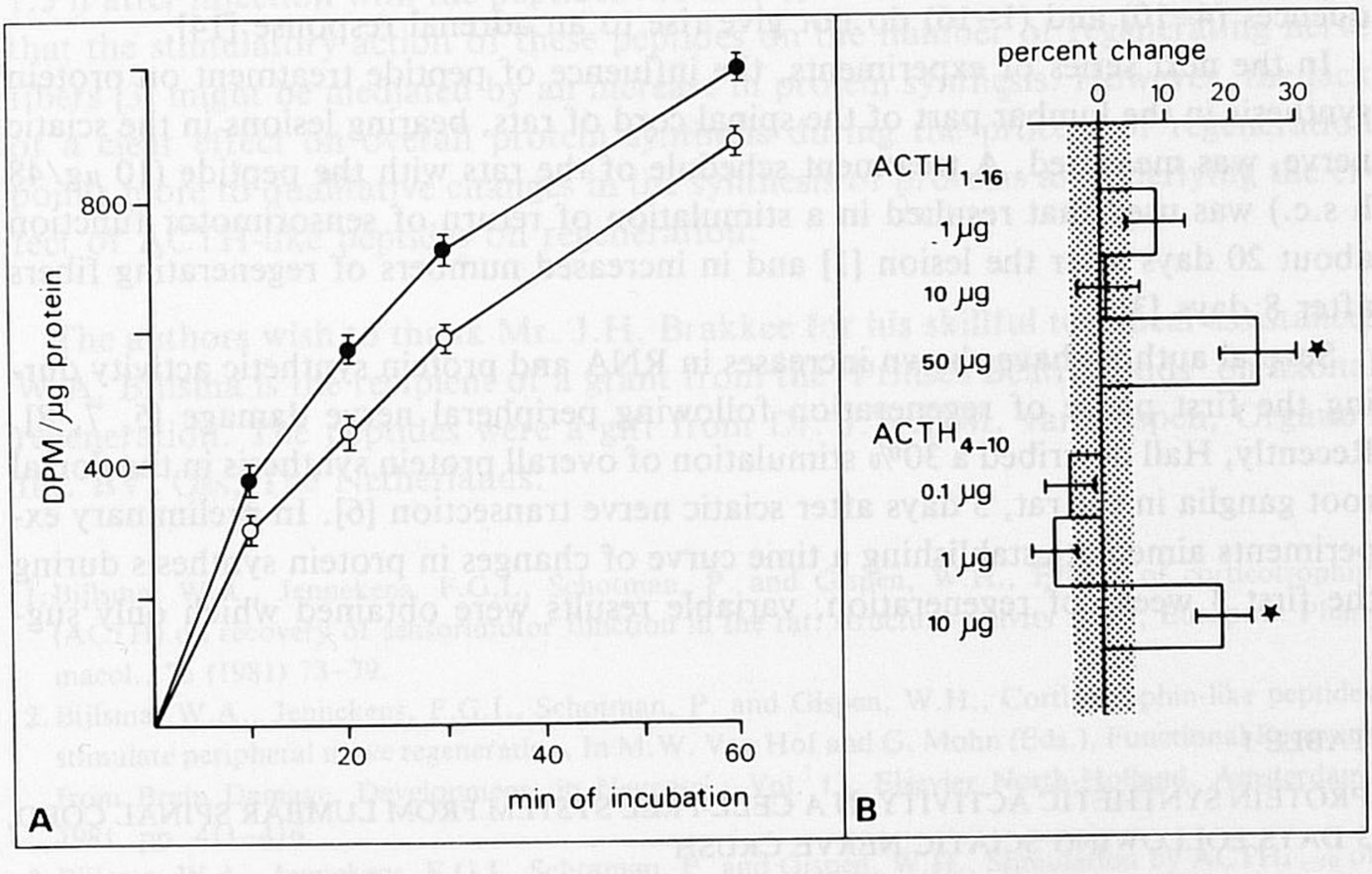

Fig. 2. Effects of injection with $ACTH_{1-16}$ or $ACTH_{4-10}$ in rats, 90 min prior to sacrifice, on the activity in cell-free protein synthesis. A postmitochondrial supernatant was prepared from the lumbar part of the spinal cord from individual animals and incubated with L-[4,5-^{3}H]leucine. A: time curves of incorporation in a preparation from saline (○—○) and $ACTH_{4-10}$ (10 μg) treated animals (●—●). B: effects of several dosages of $ACTH_{4-10}$ (at 0.1, 1.0 and 10.0 μg) and $ACTH_{1-16}$ (at 1.0, 10.0 and 50.0 μg) compared to the activity after saline treatment. Bars indicate the means of the incorporation at 10, 20, 30 and 60 min. The data were tested by a two-way analysis of variance; * $P<0.05$, n = 10.

vitro approach, the effective dosages of $ACTH_{4-10}$ and $ACTH_{1-16}$ differ with about a factor of 2 on a molar base. For both peptides, similar treatments were effective in stimulation of functional recovery after crush lesioning of the sciatic nerve [1]. Thus, systemic injections of rats with sequences of ACTH accelerate regeneration of sciatic nerve after crush and stimulate protein synthesis in spinal cord regions, enriched in the cell bodies of the motoneurons.

The question may arise as to what extent circulating ACTH levels in the saline or untreated rats, under normal or stress conditions, may affect nerve regeneration and protein synthesis. It may be expected that plasma levels of $ACTH_{4-10}$ will reach a maximum of about 50 ng/ml, 5–7 min after the injection [15]. This is, in molar concentrations, 400 times higher than in normal and 40 times more than in stress situations. However, the half-life of the injected peptide in plasma is about 4 min, resulting in a rapid decline of the level, whereas its levels in CSF may be of more relevance to the action on spinal cord protein synthesis. From behavioral studies [14], we know that peptide injections in CSF will be about 50 times as effective as in plasma (corrected for the different volumes). Thus, the single peptide dose used in these studies is at least 10 times higher than the normal circulating ACTH levels. A real comparison with the situation under stress cannot be made, because the sequences (4–10) and (1–16) do not give rise to an adrenal response [14].

In the next series of experiments, the influence of peptide treatment on protein synthesis in the lumbar part of the spinal cord of rats, bearing lesions in the sciatic nerve, was measured. A treatment schedule of the rats with the peptide (10 μg/48 h s.c.) was used that resulted in a stimulation of return of sensorimotor function about 20 days after the lesion [1] and in increased numbers of regenerating fibers after 8 days [3].

Several authors have shown increases in RNA and protein synthetic activity during the first phase of regeneration following peripheral nerve damage [5, 7, 8]. Recently, Hall described a 30% stimulation of overall protein synthesis in the dorsal root ganglia in the rat, 3 days after sciatic nerve transection [6]. In preliminary experiments aimed at establishing a time curve of changes in protein synthesis during the first 3 weeks of regeneration, variable results were obtained which only sug-

TABLE I

PROTEIN SYNTHETIC ACTIVITY IN A CELL-FREE SYSTEM FROM LUMBAR SPINAL CORD, 3 DAYS FOLLOWING SCIATIC NERVE CRUSH

The animals received injections of saline or 10.0 μg $ACTH_{4-10}$ subcutaneously, immediately and 48 h after surgery. Means of dpm/μg protein are shown ± S.E.M.; n(number of experiments) = 8. No significant changes present.

Sham-operated (saline)	Crush-operated (Saline)	Crush-operated ($ACTH_{4-10}$)
1899 ± 185	1926 ± 141	1933 ± 138

gested that 3 days after sciatic nerve crush cell-free protein synthesis in the rat lumbar spinal cord might be stimulated (unpublished data). From these data and those from the literature we decided to focus our attention on changes in cell-free protein synthesis 3 days following a bilateral sciatic nerve crush lesion with and without peptide treatment (Table I). No significant changes were observed as a consequence of crush lesioning or of ACTH treatment in crush-lesioned animals as such. Several reasons can be put forward for the lack of an effect on protein synthesis in motoneuron cell bodies after a crush lesion. One possible reason might be that the regeneration process may include a more complex reaction pattern in time and/or a shift in protein pattern for example to more synthesis of structural proteins compared to functional proteins, e.g. enzymes involved in transmitter metabolism [6, 7, 11]. In contrast, crush lesioning of the sciatic nerve resulted in a stimulation of protein synthesis in cell bodies in dorsal root ganglia [6]. However, in the case of the sensory system, the regenerating part represents a dendritic function. For that reason, the crush lesion most probably will not interfere with metabolism of transmitter enzymes [6, 7].

Summarizing, cell-free protein synthesis is increased in the lumbar spinal cord, 1.5 h after injection with the peptides $ACTH_{4-10}$ and $ACTH_{1-16}$. This may suggest that the stimulatory action of these peptides on the number of regenerating nerve fibers [3] might be mediated by an increase in protein synthesis. However, the lack of a clear effect on overall protein synthesis during the process of regeneration points more to qualitative changes in the synthesis of proteins as underlying the effect of ACTH-like peptides on regeneration.

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