Influence of Hormones on Recovery of Electrical Activity of Crushed Sciatic Nerves in Hypophysectomized Rats. (29702)

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(Introduced by Ernesto D. Salgado)
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The influence of hormones on the process of nerve regeneration has been the subject of several investigators over the past 29 years. Earlier workers reported that thyroidectomy appeared to retard nerve regeneration as determined by histological criteria and to result in delayed functional recovery; the delay in recovery was abolished by administration of thyroid extracts(2). Castration has also been reported to be advantageous for nerve regeneration(4). More recently, cortisone was found to retard significantly the regeneration rate of axons in degenerating segments, but not if treatment were initiated later than 18 days after the lesion(3). This effect was ascribed to inhibition of neurilemma cell proliferation by cortisone(5,7). Hydrocortisone acetate, however was found to markedly facilitate the rate of recovery of sensory and motor function after nerve lesion. Somatotrophic hormone (STH), on the other hand, retarded the recovery process(1). The above investigations were concerned with the histological picture of regeneration or with the recovery of kinetic activity (ambulation) as definitive criteria of regeneration. In contrast to this, a report from this laboratory(6) indicated that, in intact animals, the appearance of evoked electrical potentials distal to the site of a sciatic nerve crush following stimulation of a site proximal to the lesion was significantly accelerated by administration of STH. The present communication concerns the recovery of electrical activity in crushed sciatic nerves of hypophysectomized animals treated with various hormones.

Materials and methods. Hypophysectomized Sprague-Dawley female rats weighing 150-200 g were obtained from Hormone Assay Co., Chicago. One week after hypophysectomy, the right sciatic nerve was exposed under ether anesthesia and crushed with a smooth forceps over a 2 mm area until the nerve was translucent. The forcep was so fixed that each nerve was crushed with the same amount of pressure. The wound was then closed with wound clips and painted with Mercresin. The animals were individually caged and fed *ad libitum* a diet of Lab Blox laboratory chow and water, supplemented with oranges. Subcutaneous hormone administration was initiated 24 hours after the nerve was crushed. The daily doses of hormones given singly or in combination were as follows: 1) bovine somatotrophin, 400 μg; 2) thyroxine, 2.5 μg; 3) testosterone propionate, 2.5 mg; and 4) cortisone acetate, 2.5 mg.

At time of sacrifice, the traumatized sciatic nerve was removed, placed in a nerve box, and stimulated electrically. Action potentials were sought in the distal portion of the nerve after stimulation of the proximal portion with varying voltages. The stimulus voltages ranged from 0.5 to 150 volts; pulse duration was kept at 0.1 msec. A nerve was considered non-functional if it did not respond to stimulation with 150 volts. The resulting electrical activity was passed through cathode-loaded input stages to a Grass P6 differential preamplifier and visualized on a Dumont model 333 cathode ray oscilloscope. The electrodes were then reversed, the distal portion of the nerve stimulated, and action potentials sought in the proximal portion. In each case, presence or absence of response was recorded photographically with a Grass kymograph camera.

Since the electrical method was used only as a criterion of a degree of functional recovery, no attempt was made to analyze the recorded potentials as to the fiber spectrum or the conduction velocities indicated by the wave form. It may be noted however, that the amplitude of the recorded action potential

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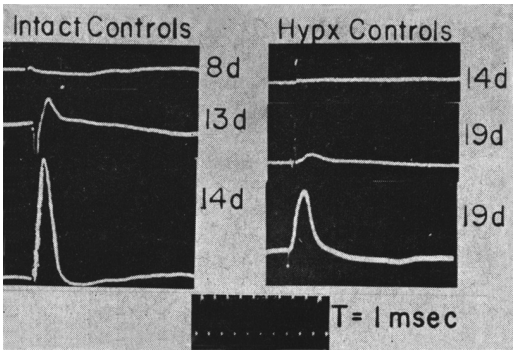

FIG. 1. Oscilloscope tracings of crushed sciatic nerves from normal intact and hypophysectomized controls which have been stimulated electrically proximal to site of lesion. Uppermost tracing shows a negative response. Middle and lower tracings represent positive evoked potential responses distal to site of injury. Electrode distance was approximately the same for all recordings. Number to the right of each tracing indicates days elapsing between nerve crush and testing.

increased with time; *i.e.*, the spike height was greater, on the average, as the interval between crush and sacrifice was lengthened. Although this observation indicates a difference in rate of recovery of nerve fibers, no attempt has been made in this study to seek a correlation between hormonal influence and the recovery rate of specific functional components in a mixed nerve.

Results. Sprague-Dawley female rats whose body weights were similar to those of the hypophysectomized animals were used to determine the recovery pattern of crushed sciatic nerve in intact animals. They were killed at regular intervals, 4 animals to a group, from the 1st to the 25th day after the nerve was crushed. Consequently, it was established that from the 14th to the 25th day of recovery, between 75 and 100% of the crushed nerves on any one particular day demonstrated action potentials distal to the lesion; recovery of electrical activity was never consistently obtained in 100% of the animals in each group. Therefore, in order to form a basis for comparison with the other studies, the first day of recovery for any one of the treated or non-treated groups was selected as the day when 75% of the animals tested demonstrated an evoked potential response distal to the site of injury. When more than 75% of the animals in a group exhibited a positive evoke potential they were represented by a solid circle in both Fig. 1 and 2.

Hypophysectomy delayed the functional recovery of nerves from the untreated animals. Action potentials were first obtained on day 18; 75% of the animals had responded by day 20, six days after the first responses were obtained from the normal control rats (Fig. 2).

Administration of hormones, either singly or in combinations, resulted in an improvement in recovery time (Fig. 2). Seventy-five percent of the nerves taken from STH-treated animals demonstrated evoked potential responses by the 9th day, as contrasted with the testosterone- and thyroxine-treated animals, whose crushed nerves responded to electrical stimulation on the 11th day. In each of the treated groups, except for the cortisone study, recovery was more rapid than that in the normal control animals. The administration of combinations of hormones in all cases resulted in further reduction in number of days of recovery time (Fig. 3); this synergistic effect was especially marked in the group receiving all hormones. Since examination of the experimental groups was started on day 6, it is not known whether some animals would have responded at earlier periods.

The results obtained with cortisone must be considered separately since never more than 64% (cortisone only) or 67% (cortisone plus STH) of the tested animals demonstrated evoked potential responses at any time. For this reason, criteria of 50% recovery have been applied in evaluating the cortisone study. On this basis, cortisone given singly resulted in more rapid recovery (14th day) than that seen in the untreated hypophysectomized animals (18th day). Recovery was the same as that obtained in the untreated intact animals (13th day) and was slower than those treated with other hormones (9th to 11th days). When combined with STH cortisone delayed by 2 days the time of recovery which had been noted when STH alone (9th day) had been administered.

Discussion. In this study, hypophysectomy was seen to retard the recovery of electrical

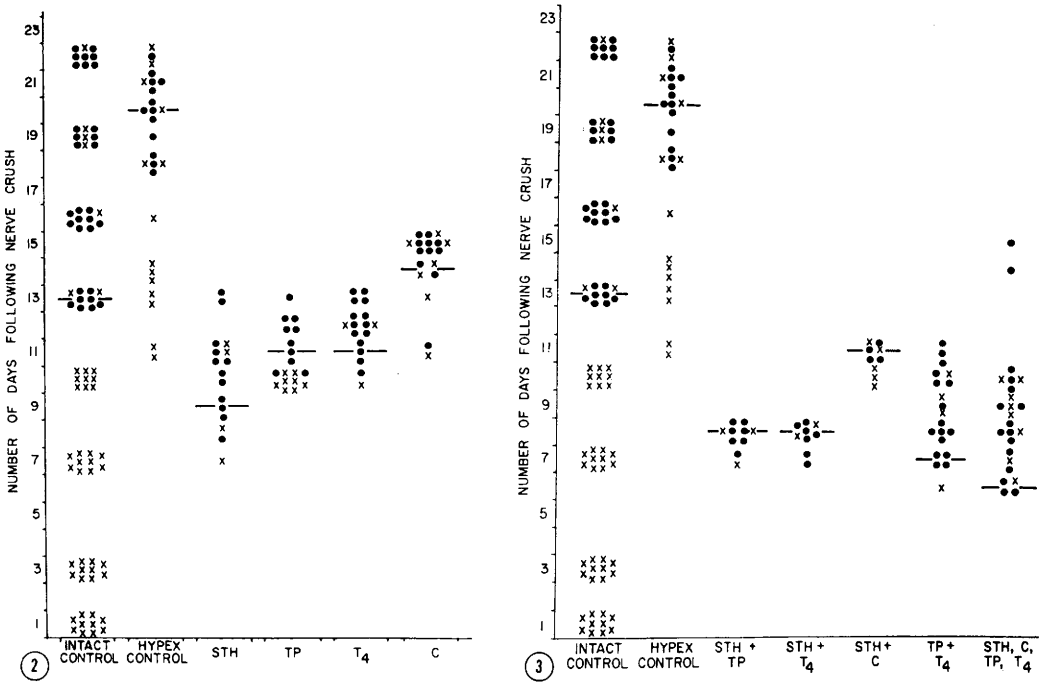

FIG. 2. Time of appearance of evoked potential responses of crushed sciatic nerves from normal animals and from hypophysectomized animals treated with various single hormones. Each symbol represents a group of 4 animals (see text). •Signifies propagation of evoked potential across lesion. × Signifies failure to elicit evoked potential. Horizontal lines indicate day on which 75% of the animals demonstrated positive responses: 50% criterion was applied to the cortisone study (see text). STH, somatotrophin; TP, testosterone propionate; T_4, thyroxine; C, cortisone acetate.

FIG. 3. Time of appearance of evoked electrical potential responses of crushed sciatic nerves from normal animals and from hypophysectomized animals treated with various combinations of hormones. Each symbol represents a group of 4 animals (see text). •Signifies propagation of evoked potential across lesion, × signifies failure to elicit evoked potential. Horizontal lines indicate day on which 75% of the animals demonstrated positive responses: 50% criterion was applied to the cortisone study (see text). STH, somatotrophin; TP, testosterone propionate; T_4, thyroxine; C, cortisone acetate.

activity of crushed sciatic nerve. The administration to hypophysectomized animals of each of the hormones investigated resulted in propagation of an action potential across the crush lesion in a reduced period of time. A synergistic effect was observed when combinations of hormones were given.

Removal of the adenohypophysis results in a generalized reduction in all metabolic functions; administration of a single hormone or of several hormones can in part or almost entirely correct the deficient metabolism of the hypophysectomized rat. Although hormones are known not to be essential for the life of the individual cells, they profoundly influence the rates of intracellular reactions concerned with protein, fat, carbohydrate, and energy metabolism. Both STH and testosterone enhance protein synthesis which is necessary to the repair of traumatized tissue, while thyroxine increases the rate of oxidative glycolysis. The presence of these hormones in tissue fluid surrounding the site of injury may directly stimulate anabolism in nerve and Schwann cells. On the other hand, the effectiveness of these hormones in correcting nutritional deficiencies by increasing dietary intake and enhancing absorption may permit the injured tissue to respond optimally to repair mechanisms. Functional recovery would then proceed in somewhat the same fashion as in the intact animal.

It is of interest that when all the hormones were administered simultaneously to hypophysectomized rats, the evoked action potential appeared by the 6th day as compared

with 14 days for the untreated normal controls and 20 days for hypophysectomized controls. We have previously reported(6) that STH exogenously administered to normal intact animals with a crushed sciatic nerve significantly improved the recovery time. Preliminary work also indicates that the same effect is obtained with other hormones. It would appear therefore, that the metabolic activity of the nerve proceeds at an optimal level and that the addition of exogenous hormones in greater than physiological amounts can accelerate these reactions that are involved in bringing about a recovery of an injured nerve.

Our data are at variance with those of Maselli-Campagna(4) that castration enhances nerve regeneration, since we have found that the exogenous administration of androgenic hormone advanced rather than retarded the recovery process. We are at a loss to explain *Bajusz'*(1) failure to find STH a beneficial agent in nerve recovery. These findings might be attributed to the subjective manner in which his data were quantitated. Certainly, STH is one of the most potent anabolic agents and one would expect to observe at least some improvement.

There is some evidence from our studies that cortisone may enhance the recovery of electrical activity of the crushed nerve; however, the recovery is not so rapid as that seen following administration of other hormones. While animals treated with STH alone demonstrated evoked potential responses by the 9th day, in those receiving cortisone concurcently with STH the responses were not obtained until the 11th day. Therefore, although cortisone has a slight beneficial effect of its own on recovery of electrical activity, a detrimental effect is evident when it is given together with STH. These findings would then agree in part with both those of Bajusz(1) that cortisone facilitates the rate of recovery and also with those of McColl and Weston(5) and of Thomas(7) that cortisone delays nerve regeneration. The explanation for this apparent discrepancy may be due to the ability of cortisone to correct disturbances in carbohydrate metabolism by gluconeogenesis while simultaneously stimulating protein catabolism. The anti-inflammatory effects of cortisone should also be considered. Inhibition of leukocytic invasion and vascularization of the area is probably detrimental to recovery, whereas prevention of subsequent fibrosis is obviously advantageous. The extent to which these effects are interrelated with those of STH would determine the response to concurrent administration of the two substances.

Summary. Hypophysectomy delayed the recovery of electrical activity of crushed sciatic nerves in adult female rats. Administration of somatotrophic hormones, thyroxine, or testosterone propionate resulted in significant improvement in rate of recovery and accelerated it beyond that seen in the intact controls. When combination of hormones were given, a further acceleration of this effect was observed. The influence of cortisone acetate was equivocal.

1. Bajusz, E., *Z. Vit. Hormon Fermentforsch.*, 1959, 10, 51.

2. Isenschmid, R., *Schweiz. Med. Wehnschr.*, 1932, v62, 785.

3. Lyitton, B., Murray, J. G., *J. Physiol.*, 1954, v126, 627.

4. Maselli-Compagna, V., *Riv. Di. Pat. Sper.*, 1935, v15, 273.

5. McColl, J. D., Weston, J. K., *Rev. Canad. Biol.*, 1953, v12, 68.

6. Soskind, L., Valdes, J., Hovde, C. A., *Anat. Rec.*, 1960, v136, 283.

7. Thomas, G. A., *J. Embryol. Exp. Morph.*, 1954, v2, 114.

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