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Effect of Spinal Cord Transection on Neuromuscular Function in the Rat

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The effect of T6 spinal cord transection on neuromuscular physiology, pharmacology, and histochemistry as well as succinylcholine-induced changes in serum potassium were studied in 88 Sprague-Dawley rats and compared to 19 control animals. Resting membrane potential of spinal cord transected animals decreased by a maximum of 15 mV. This change was significant at days 3, 7, and 30 posttransection. Cell membrane input resistance was significantly decreased at posttransection days 10 and 15. Succinylcholine-induced contracture increased 3- to 4-fold and was significantly greater than control values on days 5 and 10 posttransection. Concomitant with the development of contracture there was a spread of cholinergic receptor from the end plate region. This differed from control acetylcholine sensitivity on days 7, 15, and 30 when receptor could be detected in excess of 1000 μm from the endplate. Serum K^+ levels 3 min after administration of succinylcholine (1 mg/kg) was significantly elevated on days 10 and 30. Spinal cord transection causes denervation-like changes in the involved skeletal muscle. (Key words: Complications: hyperkalemia. Ions: potassium. Muscle: end plate. Neuromuscular relaxants: succinylcholine. Neuromuscular transmission. Receptors: cholinergic. Spinal cord: paraplegia; transection.)

THE EFFECTS of peripheral motor nerve section on the structure and function of the involved muscle have been well-described. These changes include a decrease in the resting membrane potential, alterations of membrane resistance, and spread of the postsynaptic cholinergic receptor so that it covers the entire surface of the muscle membrane.[1-3] These changes appear to be almost completely reversible if reinnervation occurs.[4]

The effect of spinal cord injury on the involved muscle has been less well studied. An early study involving the effects of an L4–5 injury on the leg muscles of the cat demonstrated cholinergic receptor spread.[5] Cord injury at this level, however, may also have inadvertently involved injury to the nerve roots supplying the muscles studied.[6] The injury and the effects seen would then be those of peripheral nerve injury, a lower motor neuron lesion rather than those of a pure spinal cord or upper motor neuron injury.[7]

Clinically, spinal cord transection has been associated with marked hyperkalemia after the intravenous administration of succinylcholine or other cholinergic agonists.[8] The chronologic relation of cord injury and the development of this response has not been well studied.

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The purpose of this study was 1) to investigate the changes in muscle function and response to cholinergic agonists following high spinal cord transection, and 2) to establish a temporal relationship for the development of these changes.

Materials and Methods

Sprague-Dawley rats weighing 200–250 g were anesthetized with diethyl ether. Thereafter a laminectomy was performed at the T6–7 level and the spinal cord was transected under direct vision. Hemostasis was obtained, the wound closed and the animals permitted to recover. One hundred twenty animals were transected and 88 animals survived and were studied and compared to 19 control animals. The completeness of the transection was evaluated in each animal prior to study by observing that the animals dragged their hind limbs and were incontinent.[9]

Studies of neuromuscular function were conducted at the following intervals posttransection: 1, 2, 3, 5, 7, 10, 15, 30, 60, and 90 days. Forty-six animals were sacrificed and both extensor digitorium longus (EDL) muscles removed. One muscle was used for microelectrode studies and the other for evaluation of succinylcholine induced contracture. The remaining 42 experimental animals and 12 controls were used to study succinylcholine-induced hyperkalemia at days 3, 5, 7, 10, 30, and 60.

The following studies were performed:

Microelectrode Studies

Miniature end plate potentials (MEPP). All experiments were carried out *in vitro* using cells from three to five EDL muscles from transected animals and compared to 28 cells from seven control rats. The preparations were mounted in a lucite bath on a paraffin-lined Plexiglas® plate in the center of which was a planoconvex lens. The muscles were stretched to about 1.25 times their resting length to minimize muscle movement. The muscles were perfused constantly with a Ringer's solution appropriately modified for the rat (NaCl 116, KCl 1.9, $CaCl_2$ 1.0, Na_2HPO_4 1.0 mM). Intracellular potentials were measured with glass capillary microelectrodes of 8–15 MΩ resistance filled with 3 M KCl in a conventional manner. Potentials were monitored on a Tektronics® 564 B storage oscilloscope and recorded on an Elema Schonander Mingograf® 81 (a polygraph having flat frequency response to 500 Hz). The time constant

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of the recording circuit was approximately 40 μs. End plate regions were located by finding MEPPs with a rapid rising phase. The MEPPs were recorded for 3 min and analyzed for frequency and amplitude. Resting membrane potential was also recorded. For these studies data from 28 control cells were compared to groups of between 11 and 28 cells in the various groups.

Acetylcholine (Ach) sensitivity. The method of assaying the effect of the ionophoretic application of Ach was similar to that employed by del Castillo and Katz.[10] The pipettes were filled with 4 M solution of Ach which was delivered by current pulses of 10-ms duration. Diffusion of Ach from the tip was prevented by the application of a direct current. Ach sensitivity was expressed as the ratio of depolarization of the membrane in millivolts to the charge in Coulombs $\times 10^{-9}$ delivered through the Ach pipette. Studies were performed on eight end plates of each of five muscles.

Action potential generation (AP). Action potentials were generated by inserting a current delivering electrode into the same cell from which membrane potential was being recorded. A depolarizing pulse was delivered through the current passing electrode and the AP so generated was photographed. These APs were analyzed for threshold for spike generation, amplitude of the spike and the rate of rise of the AP. Resting membrane potential was set at 80 mv by delivering a negative charge through the current passing electrode just before eliciting the action potentials. Action potentials were recorded and analyzed from five cells of each of two muscles in each group studied.

Input resistance. Input resistance was determined by the method of Katz and Thesleff.[11] A hyperpolarizing current was delivered through the current passing electrode and the potential change compared to the quantity of the current delivered. Five cells from each of two muscles were recorded and analyzed in each of the groups studied.

Contracture Studies

The EDL muscle was carefully removed and suspended in a temperature controlled bath of 3-ml volume. One end was firmly attached to the bottom of the bath and the other to a force displacement transducer. The muscles were stretched with 0.5 g tension and increasing concentrations of succinylcholine ranging from 0.25 to 1.0 mM were added to the bath. Succinylcholine was chosen rather than acetylcholine because it is used clinically. Viability of the muscle was tested by electrical stimulation before and after drug application. Seven normal muscles served as the control and were compared to groups of three to five muscles at the different posttransection intervals.

Potassium Efflux from Muscle

Rats were anesthetized with diethyl ether and ventilation controlled. A laparotomy was performed and the inferior vena cava cannulated for the administration of succinylcholine and the removal of blood samples. Succinylcholine, 1 mg/kg, was administered intravenously and a blood sample drawn for analysis of Na^+ and K^+ by a flame photometer. Three minutes were permitted to elapse between injection of succinylcholine and blood sampling to allow for drug distribution to the muscle and potassium efflux. Change in serum potassium level in twelve control animals were compared to those observed in groups of five to nine animals at each of the study periods.

Light Microscopy and Histochemistry

Muscles were removed from two of each of the groups of animals and subjected to light microscopy and histochemical staining for NADH (nicotinamide adenine dinucleotide) and ATP (adenosine triphosphate). Spinal cords were also removed from two animals at 30 and 60 days posttransection and studied microscopically for evidence of motor tract regeneration immediately below the transection site.

Statistics

A one-way analysis of variance was performed to examine differences among all groups for each measurement. The null hypothesis for each test is that there are no differences among groups. If this hypothesis is rejected at the 0.05 level of significance then Dunnett's test was used to determine if differences were due to differences between the control group and one or more of the treated groups. The test was two-sided with an overall level of significance of 0.05.[12]

Results

The microelectrode studies demonstrated no significant changes from the control values in MEPP amplitude and frequency. The resting membrane potential, however, did show significant alteration. In the control muscles the resting membrane potential was 69.7 ± 1.11 mV and decreased to 67.7 ± 1.8 mV ($P < 0.05$) at day 3, and to 64.7 ± 1.2 mV ($P < 0.05$) at day 7 posttransection. At day 30 the resting membrane potential had decreased further to 55.4 ± 4.8 mV ($P < 0.05$) and returned to the control value at 90 days posttransection. (fig. 1).

Action potential generation parameters demonstrated no significant change in all groups. Input resistance in control muscles was 4560 ± 163 Ω and decreased to 1129. ± 354 Ω ($P < 0.05$) at 10 days posttransection,

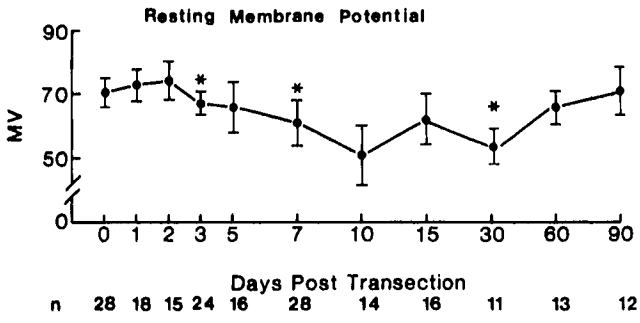

FIG. 1. EDL resting membrane potential following T6 spinal cord transection. Values are means ± SE, and n = number of cells studied. *Significant $P \leq 0.05$.

and to 2212. ± 309 Ω ($P < 0.05$) at 15 days posttransection. Input resistance was still reduced at 30 days (3291. ± 1195 Ω, $P > 0.05$).

Sensitivity of the muscle membrane to ionophoretically applied Ach was limited to the end plate region in the control group. Receptor spread was first noticed on day 3 posttransection, reached maximum in 7 days, and regressed towards normal by 90 days (fig. 2).

Muscle contracture in response to the application of succinylcholine was very slight in the control and two days posttransection muscles. On day 3 an increase in contracture was noted which was significantly different from control on days 5 and 10 posttransection and returned to close to the normal range by 30 days. In the 90-day animals there was only a slight contracture resembling that of the control animals (fig. 3).

Gross observation of the muscle demonstrated progressive atrophy that appeared to be maximal at 10 days posttransection. The control muscle dry weight was 54.91 mg ± 6.17, and was 31.43 mg ± 2.75 ($P < 0.05$) at day 10.

Light microscopic and histochemical observations of muscles of transected animals (in the first two weeks after transection) demonstrated groups of atrophic fibers. The 60 and 90 days muscles showed a return toward a normal size and cellular architecture without the usual signs of motor reinnervation of a previously denervated muscle.[4]

All spinal cords studied showed evidence of progressive axonal degeneration both proximal and distal to the site of injury. The spinal cords of the 60 and 90 day posttransection animals showed no evidence of regeneration and the area of injury was dense fibrous scar.

In the *in vivo* transected animals, the administration of succinylcholine resulted in an increase in serum potassium levels starting on day 3. The change in serum K^+ levels was significantly different from control value ($P < 0.05$) at days 10 and 30 posttransection. There was then a gradual return toward the normal value by day 60. There was no significant change in the serum sodium concentration (fig. 4).

Discussion

The development of contracture in skeletal muscle in response to the *in vitro* application of Ach or a cholinergic agonist is a phenomenon seen in a number of instances. It is seen as a normal occurrence in amphibians or avian neck muscle. To a lesser extent it can be seen in slow or red mammalian muscle. In our studies a small degree of contracture was noted in the control EDL muscle. This muscle, though predominantly white or fast in character, does have a small percentage of slow or red fibers and it is the interaction of the cholingeric agonist with these that produces the small amount of contracture seen in the control circumstance.[4] Contracture is also seen in muscle after peripheral nerve section. The development of contracture after peripheral nerve section appears to be related to the spread of cholinergic receptor over an extended area of the muscle membrane. The increase in magnitude of contracture in response to cholinergic ag-

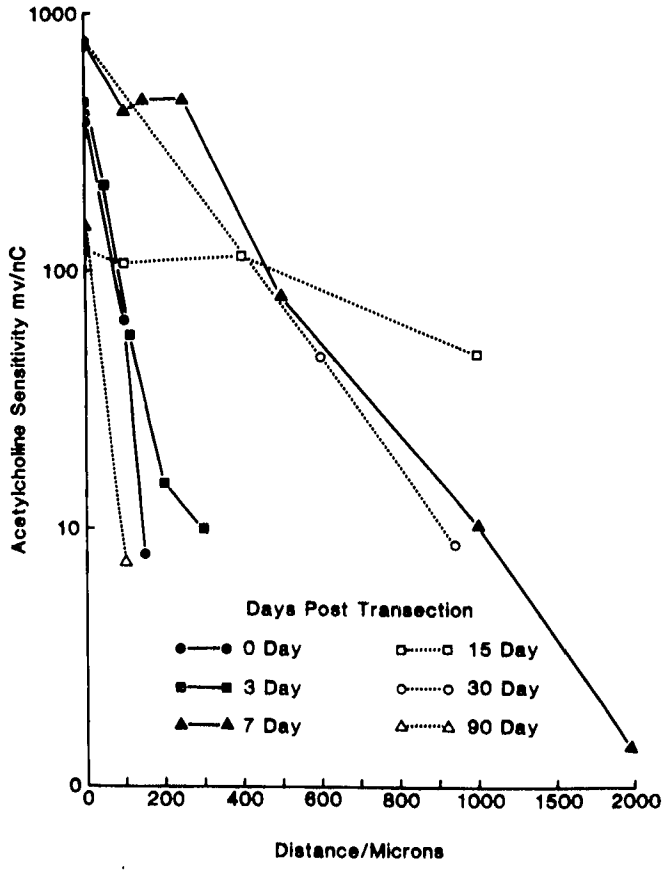

FIG. 2. A graphic representation of cholinergic receptor spread in the EDL following spinal cord transection. Each line depicts extent of cholinergic receptor in one cell at the specified time.

onist seen in our studies suggests that the spinal cord transection also causes spread of cholinergic receptor.

The cholinergic receptor spread is further documented by our ionophoretic studies. In normal fast muscle, cholinergic receptor is closely localized to the postsynaptic region and depolarization in response to the ionophoretic application of Ach can be noted only within about a 100–150 μm radius of the end plate. Beginning at day 3 following transection of the spinal cord, cholinergic receptor was noted to have spread out from the end plate region. This spread reached maximum in about one week and then regressed toward normal by 90 days. The data presented in the ionophoretic study represent only single cells at the times listed in figure 2. This procedure is technically difficult since it requires localizing an end plate region which is only about 20–30 μm in diameter.[13] This localization is made even more difficult and imprecise when cholinergic receptor has spread out from the end plate region and accounts for the rather broad range of focal Ach sensitivities seen in figure 2. It is also complicated by the atrophy of the muscle fibers which is noticeable by the end of the first week.

Coincident with the development of contracture in response to cholinergic agonist and the observed spread of cholinergic receptor noted by ionophoresis was an increase in serum potassium following the intravenous administration of succinylcholine.[6] This implies that the receptor and the opening of membrane channels permit the efflux of potassium from the muscle to the extracellular fluid and eventually the blood. All of these observations appear to have returned to about the normal state by three months posttransection.

The underlying mechanism for the spread of cholinergic receptor after peripheral nerve or spinal cord transection is unknown. Blockage of fast axonal transport by colchicine has been noted to cause the same phenomenon.[14] Additionally, there now exits experimental evidence in animals that both prolonged immobilization[15] and the continued administration of neuromuscular blocking agents[16] also cause receptor spread. The occurrence of hyperkalemia following the administration of succinylcholine to experimental animals after prolonged immobilization has been noted to cause increased potassium efflux from muscle.[17] The effect of prolonged *d*-tubocuraine-induced paralysis on potassium efflux has not been studied.

The present study supports the observations of Johns and Thesleff[5] of the development of a denervation-like phenomenon following spinal cord transection. As previously noted, these authors performed their cord transections at a lumbar level and therefore might have injured the peripheral nerves with a resultant denervation effect on the muscle. We performed our transection at the midthoracic level to avoid this problem. To specifically rule out the possibility of denervation we looked for histochemical signs of reinnervation. These are the observations of large groups of NADH staining cells in the reinnervated muscle compared to their usual diffuse pattern in the normal muscle. The changes in muscle were first noted at three days posttransection, peaked at 10 to 15 days, and then regressed toward normal by 60–90 days. The hyperkalemic response to systemic succinylcholine administration correlated with the changes in the muscle.

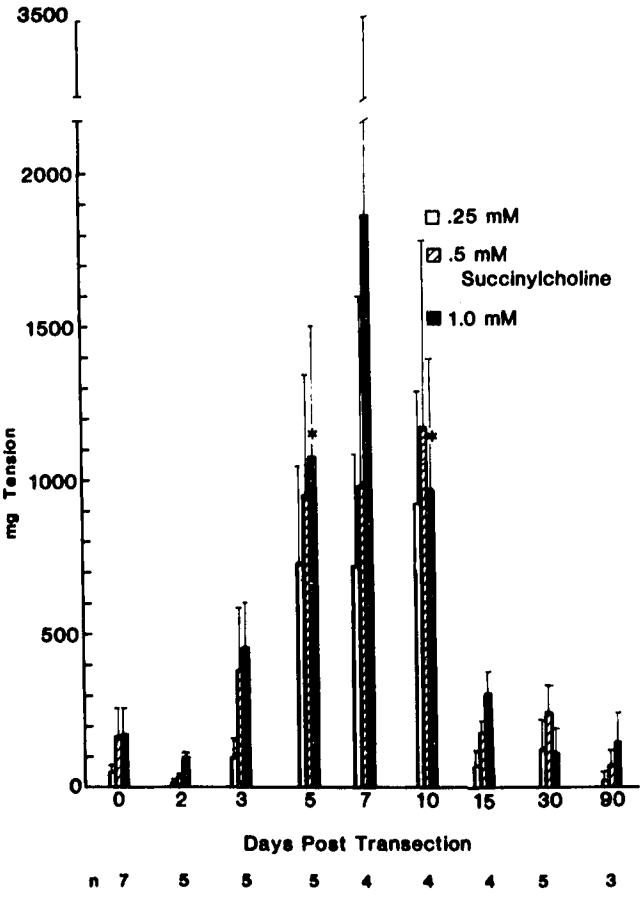

FIG. 3. EDL contracture in mg with 0.25, 0.5, and 1.0 mM succinylcholine in relation to time elapsed following spinal cord transection. Values are means ± SE, and n = number of muscles. *Significant $P \leq 0.05$.

The occurrence of a temporary atrophy followed by a return of muscle bulk has been noted previously in spinal cord transected rats.[7] It could be postulated that the atrophy, cholinergic receptor spread, and increased potassium efflux might be due to temporary anterior horn cell dysfunction below the level of transection as part of a spinal shock phenomenon. With the resumption of spinal cord reflex activity at the termination of the period of spinal shock, one might see a return of muscle bulk, regression of receptor spread, and a disappearance of the abnormal potassium efflux. This agrees with the pre-

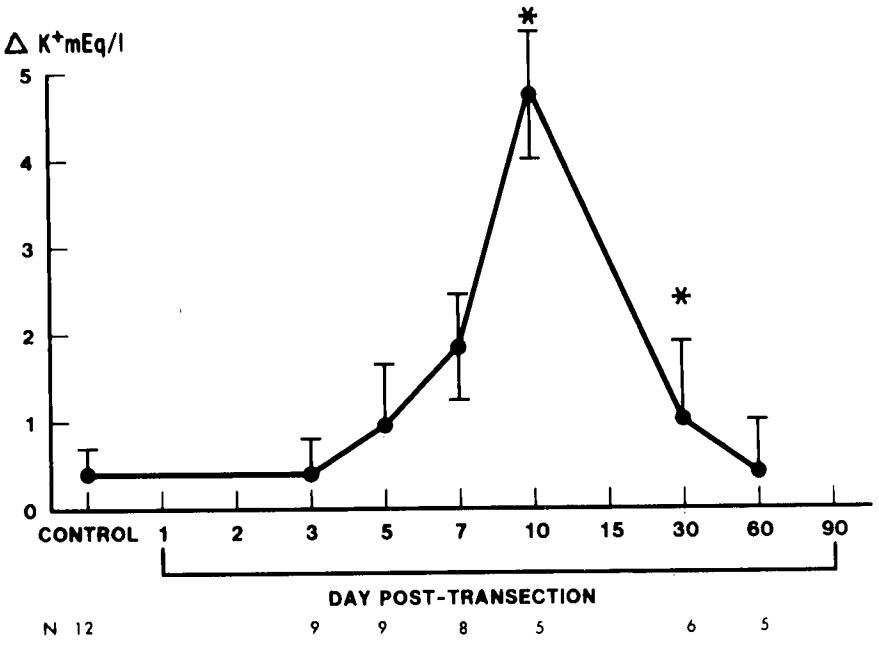

FIG. 4. K^+ ion increase in response to 1.0 mg/kg succinylcholine *in vivo* following different periods of spinal cord transection. Values are means ± SE, and n = number of animals. *Significant $P \leq 0.05$.

vious study[7] and suggests that the period of spinal shock in the rat is approximately two weeks.

Although these studies were conducted in the rat, they correlated well with clinical observations in humans.[18] On this basis, there should be a safe period of at least 48 h for the administration of succinylcholine following spinal cord transection without the development of critical levels of hyperkalemia. Because of the very apparent difference in the response of the muscle of the rat compared to humans, we are unable to make a definitive statement regarding the duration of the hyperkalemic response in humans.

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