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Electrophysiological investigations of the mild spinal cord injury in rats

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Abstract. Somatosensory evoked potentials (SEPs) waveform recorded from the normal and injured spinal cord in rats were analyzed. A mild compression spinal cord injury was performed in two experimental models. The first model was used to assess the disappearance and the second one the return of the electrophysiological spinal cord function after injury. In the first experimental model typical changes in waveforms morphology from normal to isoelectric line during spinal cord compression were investigated. In the second model an isoelectric line was achieved by more severe but a shorter time of compression and the typical changes in SEPs morphology after decompression were described. Our evidence suggests that the spinal cord function disappears gradually during mild compression and can return early after decompression. The amplitude after decompression first improved and then gradually deteriorated which is probably caused by secondary insult. Experimental models are both effective and simple and may be used to evaluate the effect of treatment of spinal cord injuries.

Key words: spinal cord compression, spinal cord decompression, SEPs, rat

INTRODUCTION

The purpose of our investigation was the assessment of the spinal cord function on rats and to produce an experimental model when spinal cord function can return after mild compression. Severe, irreversible spinal cord injury was relatively well recognized on various experimental models (Tator 1991). Mild spinal cord injury should be better investigated because that pathology can be treated more successfully. The study of changes in the compressed spinal cord allow to select the best drugs which can protect spinal cord function. Investigations of electrophysiological changes during mild spinal cord compression have clinical implication in electrophysiological monitoring of spinal cord function during neurosurgical operations on spinal cord and can also decrease the risk during these operations (Kamimura et al. 1988, Daube 1989, Nishijima at al. 1992, Koyanagi et al. 1993). Evoked potential monitoring for electrophysiological assessment of spinal cord integrity is an increasingly important tool in patients who have, or are at risk of developing spinal cord injury, and in the laboratory study of spinal cord injury (Hurlbert at al. 1992). The use of neurophysiological tests outcome measures in experimental spinal cord injury has contributed greatly to our understanding of the pathophysiological mechanisms and has provided easily applied and accurate tests of function for assessing the severity of spinal cord injury and the response to treatment. Spinal evoked potentials, motor and somatosensory evoked potentials and evoked potentials elicited by stimulation of the cerebellum have been the most useful (Tator 1991). The somatosensory evoked potentials investigation was chosen in our experimental model because this electrophysiological, safe method is frequently used in clinical practice since Cracco (1973) had recorded spinal SEPs. Recently SEPs are frequently used as the sole method of monitoring in the operating room (Friedman 1986). The spinal cord patways that conduct these evoked responses involve primarily the dorsal columns with some contribution, during lower limb stimulation, from the dorsal spinocerebellar tract.

METHODS

Subject and operative procedures

Thirty adult male Wistar rats (weight 330-450g) were operated under general anesthesia with an intraperitoneal injection of chloral hydrate 4% solution using a dose of 0.9 ml / 100g of body weight and a local injection of 1% lignocaini. Rats were placed on a warming animal operating table and a rectal temperature was maintained at 36-37 ^{o}C. The left and right sciatic nerves were exposed above the knee and electrodes, consisting of two fine stainless steel needles separated by 1.5 mm were inserted into the muscle, in contact with the sciatic nerve.

A laminectomy at the Th9 - Th10 level was performed under operating microscope using a dental drill. The SEPs were recorded before and after laminectomy. Animals with the same SEPs pattern before and after laminectomy were blindly allocated to one of two groups (fifteen rats in each) differing in the type of spinal cord compression. The simply apparatus for precise measurement of spinal cord compression was constructed. This apparatus produce a blocking - weight type of compression. In the first group the weight of 14.8g was applied on the spinal cord extraduraly for 60 min. The SEPs were monitored during all the time of compression. The time when isoelectric line appeared was assessed by two independent investigators. In the second group the weight of 19.8 g was applied on the spinal cord extraduraly for about 10 min. until the appearance of the isoelectric line. Then the weight was removed. The SEPs were monitored during compression and during 1 h after decompression . A deterioration of the spinal cord function was assessed in the first model and an improvement of the spinal cord function was assessed in the second model.

SEPs monitoring procedures

The SEPs were recorded on a Spirit (Nicolet) signal averager. Both sciatic nerves stimulation resulted in cortical responses. A total of 250 stimuli were averaged after stimulation. Anoda and kathoda Ag:AgCl disc electrodes 3 mm in diameter were placed subcutaneusly on the skull bone over the sensorimotor cortex. Impulses to sciatic nerves were delivered with an intensity of 1-4 mA, a duration of 0.2 ms and frequency 3.7 Hz. The bandpass was 30-3.000 Hz .

Measured parameters and statistical analysis

The latency of the 5 peaks N1, P1, N2, P2 and N3 were measured before laminectomy in all (30) animals and then were analyzed. These data were used to determine the standards of the SEPs latency in intact rats.

In the first group the time when the isoelectric line appeared was determined.

In the second group the following parameters were analyzed:

1. The time when the highest amplitude N1P1 returned after decompression.

2. The value of amplitude N1P1. The following comparisons were made: between the waveform recorded after laminectomy and the waveform with the highest amplitude N1P1 recorded after decompression, between the waveform recorded after laminectomy and the waveform recorded 60 min after decompression, between the waveform with the highest amplitude N1P1 and the waveform recorded 60 min after decompression.

3. The latency of N1 and P1 in the waveform after laminectomy and in the waveform recorded 60 min after decompression. The comparisons of amplitudes N1P1 and of their latencies were made using the Wilcoxon test. This tests was chosen because the analize data did not have normal disturbation (Podgórski 1996).

In each group the ranges, the average value, the median values and the standard deviations of each parameter were determined.

RESULTS

In intact rats typical SEPs pattern under anesthesia and before laminectomy consists of repeatable two positive peaks P1, P2 and three negative peaks N1, N2, N3 (Fig. 1). Thirty rats had recorded SEPs pattern before laminectomy and the latency of N1, P1, N2, P2 and N3 were measured. The obtained values are shown on Table I.

In the first experimental group the isoelectric line was observed in all (15) animals during 60 min compression.

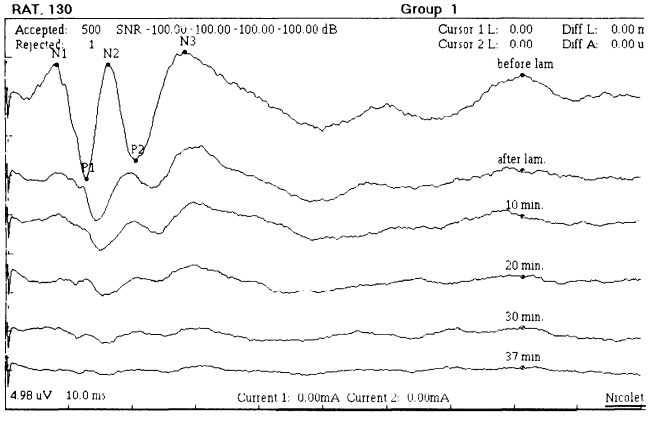

Fig. 1. The typical rats SEPs waveform pattern under anesthesia and before laminectomy. Both waveforms were recorded in rat number 113. Two positive (P1, P2,) and three negative (N1, N2, N3) peaks are indicated.

TABLE I

The values of the latency (in miliseconds) of particular peaks (N1, P1, N2, P2, N3) in SEPs in normal rats

	N1	P1	N2	P2	N3
Number of cases	30	30	30	30	30
Max value	12.0	17.2	23.0	25.4	37.2
Min value	8.2	12.8	16.2	20.6	28.2
Average	9.8 ± 1.1	14.8±1.4	19.9±1.6	24.7±1.7	32.7±2.8
Median	9.4	14.4	19.8	24.6	32.8

The time taken for the isoelectric line to appear were as follows: minimal and maximal time 13.0 and 32.0 min. respectively, average time: 21.3 min., SD: 6.9, median: 20.0 min.

The rats SEPs waveform peaks disappeared in a strictly defined way. Peaks with long latency (N3, P2, N2) disappeared more quickly while peaks with early latency (N1, P1) were the most stable (Fig. 2).

In the second experimental model all 5 peaks which were determined before compression appeared at precisly the same moment after decompression (Fig. 3).

The time when the best waveform (the waveform with the highest amplitude between peaks N1P1) returned after decompression in the analyzed 15 rats was as follows: minimal and maximal time 15.0 and 47.0 min. re-

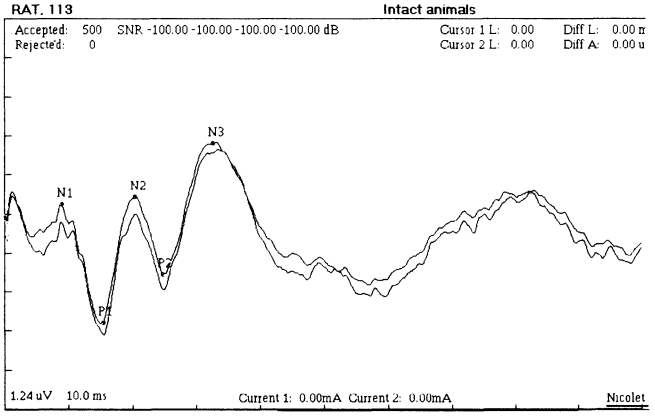

Fig. 2. Example of the rat SEPs waveform pattern from the first group. The first waveform was recorded before laminectomy, the second was recorded after laminectomy, the third - 10 min compression, the fourth - 20 min compression, the fifth -30 min compression. The last waveform - the isoelectric line, was recorded in 37 min of compression.

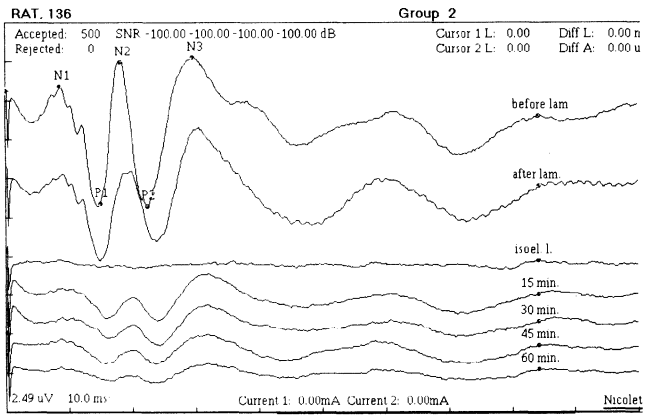

Fig. 3. Example of the rat SEPs waveform pattern from the second group. The first waveform was recorded before laminectomy, the second was recorded after laminectomy, the third (isoelectic line) was recorded after 10 min compression, while the rest were recorded every 15 min after decompression. Note that the waveform amplitude improved after decompression and then gradualy deteriorated. The last waveform recorded 60 min after decompresion had reduced amplitude than the waveforms recorded 15, 30, 45 min after decompression respectively.

spectively, average time: 25.1 min, SD: 10.4, median 19.0 min.

The value of amplitudes N1P1 were statistically analysed in the second experimental group (Table II). The

TABLE II

The value of amplitudes N1P1 in the second experimental group (in microvolts)

	Waveform recorded after laminectomy (I)	Waveform with the highest amplitude after decompression (II)	Waveform recorded 60 min after decompression (III)
Number of rats	15	15	15
Max. value	8.8	6.5	3.4
Min. value	2.1	1.2	0.8
Average value ± SD	4.9 ± 1.8	3.0 ± 1.2	1.5 ± 0.7
Median	5.3	3.0	1.3
Results of statistical analysis	$W_{I/III} = 3.94$ $P<0.001$	$W_{I/II} = 3.84$ $P<0.001$	$W_{II/III} = 4.10$ $P<0.001$

TABLE III

The latency of N1 in the second experimental group (in milisecond)

	Waveform recorded after laminectomy	Waveform recorded 60 minutes after decompression
Number of rats	15	15
Max. value	11.9	13.1
Min. value	8.0	8.3
Average value ± SD	9.6 ± 1.2	10.5 ± 1.3
Median	9.5	10.5
Results of statistical analysis	W = 3.65	$P<0.001$

N1P1 amplitudes measured on the waveform recorded after laminectomy before compression were significant higher compared with every waveform recorded after decompression. The highest amplitudes were measured about 25 min. after decompresion. After that the significant deterioration of value N1P1 amplitudes were observed.

In the second experimental group the latency of peaks N1 and P1 were measured on the waveform recorded

TABLE IV

The latency of P1 in the second experimental group (in milisecond)

	Waveform recorded after laminectomy	Waveform recorded 60 min after decompression
Number of rats	15	15
Max. value	16.5	16.8
Min. value	12.8	13.1
Average value ± SD	14.7 ± 1.1	15.2 ± 1.3
Median	15.1	15.8
Results of statistical analysis	W = 3.44	$P<0.001$

after laminectomy and compared statistically using the Wilcoxon test with latency measured on the waveform recorded 60 min after decompression (Table III and IV). The changes in N1 and P1 latency were significant and showed increase at 60 min after decompression.

DISCUSSION

Since Allens (1911) classical work , many investigators have been concentrating on the problem of the experimental spinal cord injury. Various experimental models have been developed to study the spinal cord injury because there is not a single ideal experimental injury model just as there is no stereotypical clinical spinal cord injury (Anderson and Stokes 1992). The goals and objectives of the research dictate specific requirements of the model. We chose the model of mild compression caused by application of weight because we wanted to record continuously the spinal cord function by SEPs and this model seemed to us the most convincing. The use of neurophysiological method to test outcome measures in experimental spinal cord injury has contributed greatly to our understanding of pathophysiological mechanisms and has provided easily applied and accurate tests of the spinal cord functions. Motor evoked potential (Fehlings at al. 1987, Wang at al. 1993), somatosensory evoked potentials (Schramm at al.1979) and evoked potentials elicited by stimulation of the cerebellum (Hurlbert at al. 1992, 1993) have been the most useful. Cortical SEPs were more sensitive to mild spinal cord injury than motor evoked potentials (Zileli and Schramm 1991). Our experimental model proved that SEPs monitoring is a very sensivity method for assessing the spinal cord function.

The latency standards of the SEPs in intact rats were determined what is important for another experiments on rats spinal cord function. Our first experimental model of mild spinal cord compression allowed to assess the evolution of postraumatic spinal cord function changes and therefore this model can be used to evaluate the efficiency of a treatment protocol. Abolition of the spinal cord function, which is demonstrated as the isoelctric line in SEPs, was reversible. This was proved from the second experimental model of mild spinal cord compression.

The amplitude and latency of peaks change during mild spinal cord compression.

This very important observation was made on the second experimental group. We noted that SEPs amplitude improved quickly after decompression and was the highest at about 25 min after decompression and then gradually decreased. This phenomenon was statistically significant. In our opinion this disturbance of decompressed spinal cord function is caused by secondary insult. There is an electrophysiological evidence that there are two mechanisms of damage to the spinal cord after injury, the primary mechanical injury (acute compression, impact, missile, distraction, laceration) and a secondary injury due to one or more additional damaging processes initiated by primary injury (Tator 1991). The secondary injury mechanisms includes vascular changes, electrolyte changes, biochemical changes, edema and loss of energy metabolism (Janssen and Hansebout 1989, Tator and Fehlings 1991). These changes were called as postraumatic secondary ischemic insult.

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