

## Effects of a Calcium Channel-Blocking Agent, Nimodipine, on Injured-Spinal Cord Na<sup>+</sup>-K<sup>+</sup>- ATPase Activity

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**Abstract** : Five minutes before spinal cord injury, an 0.02 mg/kg dose of nimodipine was started via a femoral catheter and continued for 96 hours after injury. Pre-and post-injury and post-infusion mean arterial blood pressure (MABP) recordings were obtained. The levels of injured cord sodium-potassium-adenosine triphosphatase activity were measured. There was a statistically significant decrease in MABP after cord injury and also a small but not significant increase in MABP after the beginning of nimodipine infusion.

The levels of injured cord Na<sup>+</sup>-K<sup>+</sup>- ATPase activity in control and nimodipine-treated groups showed no statistically significant difference ( $p>0.05$ ).

The results indicated that nimodipine has no effect related to the Na<sup>+</sup>-K<sup>+</sup>- ATPase activity on the cell membrane in injured spinal cord.

**Key Words** : Experimental spinal cord, Na<sup>+</sup>-K<sup>+</sup>- ATPase, nimodipine, rat.

### INTRODUCTION

Calcium may play a central role in various pathological ischaemic states (7). Measurements of spinal cord blood flow after spinal cord injury have shown ischemia at the injury site and extending considerable distances (8). In some experimental studies, the beneficial effects of pharmacotherapy on spinal cord injury have been reported with calcium antagonists (2,5,8,14).

Na<sup>+</sup>-K<sup>+</sup>- ATPase is essential for cellular events because it is embedded in the cell membrane (6,9,10,12). It has been demonstrated that the activity of Na<sup>+</sup>-K<sup>+</sup>- ATPase is a useful parameter for evaluating cellular disturbance by ischemia in order to determine the possible therapeutic effect of any agent (6).

In this study, we investigated the alterations of Na<sup>+</sup>-K<sup>+</sup>- ATPase activity after spinal cord injury with or without nimodipine treatment.

### MATERIALS AND METHODS

Ten male Wistar rats, weighing 250-330 gm each, were used for this study. All surgical procedures were performed under anaesthesia induced by intraperitoneal ketamin hydrochloride (50 mg/kg). Animals were divided into two groups: 5 in the control and 5 in the experimental group. The left femoral artery and right femoral vein were cannulated, the femoral vein for nimodipine infusion and the femoral artery for measuring blood pressure.

A three-level laminectomy from T5 to T8 was performed under an operating microscope. The clip compression injury model was used and each rat received a 1-minute extradural clip-compression injury of the cord with an aneurysm clip exerting a force of 50 g (Yaşargil Temporary Clip, Aeusculap, West Germany).

In the experimental group animals, five minutes before spinal cord injury, nimodipine infusion at a rate of 0.2 g/kg/min (2,5,8) was started via the

femoral catheter. In the control animals, normal saline solution was infused at the same time and rate as the nimodipine. This infusion was continued for 96 hours after injury. In all animals, blood pressure monitoring was terminated 1 hour after the injury.

The animals were sacrificed, the spinal cord was removed and 1 cm samples were taken from the injury site and stored at -70°C for assay of Na<sup>+</sup>-K<sup>+</sup>-ATPase activity.

**Assay of sodium-potassium-adenosine triphosphatase activity**

Each frozen spinal cord was divided into two parts longitudinally. The divided cord tissue was homogenized in 5 ml of 25 mmol/L ice-cold Tris HCL buffer (pH, 7.4) containing 0.32 mol/L sucrose. The homogenate was incubated at 37°C for 10 minutes in the presence of 100 mmol/L NaCl, 30 mmol/L KCl, 25 mmol/L MgCl<sub>2</sub>, and 3 mmol/L 2Na-adenosine triphosphate. Inorganic phosphate was measured by the method of Fiske and Subbarow. Na-K-ATPase activity was calculated as the ouabain-sensitive ATPase activity and expressed as mol Pi/mg protein/10 minutes. Protein in the homogenate was measured by the method of Lowry. Mean activity in the injured spinal cord as a whole was calculated from mean results determined in two parts of each injured cord.

**RESULTS**

**Physiological Parameters**

The mean preinjury MABP (115.7±5.9 mmHg in control, 112.4±7.1 mmHg in experimental group) and heart rate (482±28 beats/min. in control, 451±32 beats/min. in experimental group) were not significantly different (p>0.05) between two groups. After spinal cord injury, there was a significant decrease in MABP in all animals. This difference was statistically significant (p>0.05). Following infusion of nimodipine, the MABP increased (p>0.05) but at 1 hour after infusion was similar in the groups (p>0.05) (Table 1, Fig. 1).

**Na<sup>+</sup>-K<sup>+</sup>-ATPase Activity**

Mean Na<sup>+</sup>-K<sup>+</sup>-ATPase activity in spinal cord was 0.329±0.106 M Pi/mg protein/10 min in nimodipine-treated animals (Table 2). No significant difference in the Na<sup>+</sup>-K<sup>+</sup>-ATPase Activity levels was found between these two groups (p=0.310).

	MABP (mmHg)	Heart Rate (beats/min)
pre-injury placebo-saline	115.7±5.9	482±28
pre-injury nimodipine	112.4±7.1	451±32
post-injury placebo-saline	83.9±6.8	502±31
post-injury nimodipine	92.0±5.2	492±21
post-infusion 5.min placebo-saline	83±8.2	513±38
post-infusion nimodipine	94.1±5.8	483±29
post-infusion 1.hr placebo-saline	98.4±7.4	494±22
post-infusion 1.hr nimodipine	92.4±7.9	454±32

hr = hour, MABP = mean arterial pressure

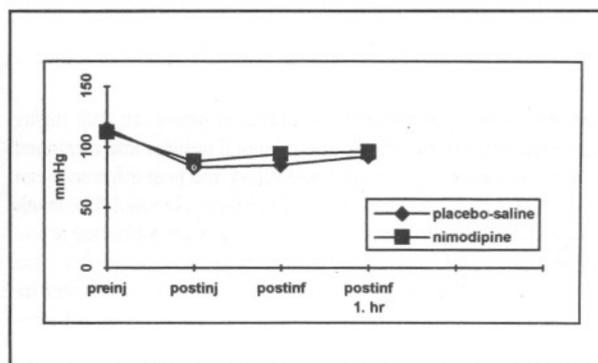


Fig. 1 : Graph showing the MABP in both groups. Note that there was significant decrease in MABP after cord injury in both groups and an increase in the nimodipine-treated group.

	no. of rats	Na <sup>+</sup> -K <sup>+</sup> -ATPase Activity ( mol Pi/mg protein/10 min.)*
Control group	5	0.329±0.026
nimodipine-treated group	5	0.27±0.106

\* = There is no statistically significant difference (p=0.310)

**DISCUSSION**

In one experimental study, it was demonstrated that the 0.05 mg/kg dose of nimodipine caused a 25 % reduction in mean arterial pressure and an 0.10 mg/kg dose caused a 37 % reduction (7). It was also demonstrated a decrease in MABP with the infusion of 0.02 mg/kg nimodipine and dextran 40 (2). Fehlings, et al., obtained identical results in their study after nimodipine infusion (5). Guha, et al., in their

study, noted a decline in MABP after injury but did not demonstrate a reduction after spinal cord injury and a small increase following nimodipine infusion (Table 1, Fig. 1). This increase was not statistically significant ( $p > 0.05$ ).

The exact mechanism of nimodipine on spinal cord injury is not fully known (14). It is postulated that the intrinsic properties of CNS vessels, such as their predominant dependence on extracellular calcium for contraction, the pharmacological properties of nimodipine, including permeation of the blood-CNS barrier or receptor specificity in the CNS might play a role (7,13,16). Demopoulos, et al., reported that cell membrane damage in the central nervous system following cerebral ischaemia and spinal cord injury may be induced by free-radical reaction and lipid peroxidation (4). These processes are energy-dependent events. Na<sup>+</sup>-K<sup>+</sup>- ATPase directly or indirectly controls many essential cellular functions, including cell volume, free calcium concentration and membrane potential (15). Clendenon, et al., reported that the activity of this enzyme decreased as early as 5 minutes after spinal cord injury in dogs (3,11). One study also demonstrated the relationship between the inhibition of activity of this enzyme and lipid peroxidation following spinal cord injury (1). In our investigation, after spinal cord injury, nimodipine treatment at a rate of 0.2 gr/kg for 96 hours had no effect on Na<sup>+</sup>-K<sup>+</sup>- ATPase activity in the injured spinal cord.

In conclusion, these results demonstrated that nimodipine has no stabilizing effect related to Na<sup>+</sup>-K<sup>+</sup>- ATPase activity on the cellular membrane in spinal cord injury.

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