Neurol Med Chir (Tokyo) 36, 296~299, 1996

Effect of Thyrotropin-releasing Hormone on Na^+-K^+-Adenosine Triphosphatase Activity Following Experimental Spinal Cord Trauma

Süleyman BAYKAL, Savaş CEYLAN**, Haydar USUL, Fadıl AKTÜRK, and Orhan DEĞER*

*Departments of Neurosurgery and *Biochemistry, School of Medicine, Black Sea Technical University, Trabzon, Turkey; **Department of Neurosurgery, School of Medicine, Kocaeli University, Kocaeli, Turkey*

Abstract

The effect of thyrotropin-releasing hormone (TRH) on spinal cord Na^+-K^+-adenosine triphosphatase (Na^+-K^+-ATPase) activity after spinal cord injury was evaluated in rats. The rats were injured by compression of the cord at T-10 for 1 minute with a 50-g clip. Saline in the placebo group (n = 8) and TRH (0.6 mg per dose) in the TRH group (n = 9) were administered intraperitoneally as bolus injections in two doses, at 45 and 120 minutes after the injury. The Na^+-K^+-ATPase activity level in the TRH group was significantly higher (p = 0.024) than in placebo group. These results indicate a possible role for TRH treatment in spinal cord injury.

Key words: Na^+-K^+-adenosine triphosphatase, spinal cord injury, thyrotropin-releasing hormone

Introduction

Spinal cord injury is a very distressing problem for patients and relatives, and imposes a large burden on society. The pathophysiology of acute spinal cord injury has not been fully elucidated. There is histological and physiological evidence that the initial primary injury, the mechanical deformation, and/or the destruction of neurons may be progressively worsened by secondary injury. Pharmacological intervention in spinal cord injury aims to interrupt the secondary mechanisms, including ischemia, lipid peroxidation, calcium-mediated cellular toxicity, free oxygen radicals, and other secondary injury effects.

Cell damage following ischemia or cord injury may be induced by free-radical reaction.[9,19] Some membrane-bound enzymes require phospholipids for maintenance of their activities, such as Na^+-K^+-adenosine triphosphatase (Na^+-K^+-ATPase) which is very susceptible to free-radical reaction and is essential for cellular excitability and very responsive to ischemia.[19] In our previous studies, we showed that thyrotropin-releasing hormone (TRH) has a beneficial effect on the early posttraumatic histological scores and electrophysiological function.[6,7]

The present investigation attempted to confirm biochemically the favorable effect of TRH in a rat model of spinal cord injury by measuring changes in Na^+-K^+-ATPase activity.

Received June 28, 1995; Accepted November 27, 1995

Materials and Methods

Seventeen Wistar rats, weighing 225–300 g, were anesthetized with ketamine hydrochloride (100 mg/kg intraperitoneally) and the femoral artery and vein were cannulated for monitoring mean arterial blood pressure (MABP) and obtaining blood samples and fluid infusion. Using the operating microscope, a four-level laminectomy was performed from T-9 to T-11. Extradural clip compression of the spinal cord at T-10 for 1 minute was performed in all rats using Yaşargil minianeurysm clips (Aesculap AG, Tuttlingen, Germany) exerting a force of 50 g.

The animals were separated into two groups: place-

bo (8 rats) and TRH groups (9 rats). Saline in the placebo group and TRH (0.6 mg per dose) in the TRH group were administered intraperitoneally as bolus injections in two doses, at 45 and 120 minutes after the injury.

Animals were sacrificed at 24 hours after injury. The spinal cord was removed and 1-cm samples were taken from the injury site. The sample was stored in a −70°C freezer before assay of Na^+-K^+-ATPase.

Each sample of frozen spinal cord was divided into two longitudinal strips. The cord tissue was homogenized in 5 ml of 25 mol/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 mol/l NaCl, 30 mol/l KCl, 25 mol/l $MgCl_2$, and 3 mol/l disodium-adenosine triphosphate. Inorganic phosphate was measured by the method of Fiske and Subbarow.[13] Na^+-K^+-ATPase activity was calculated as the ouabain-sensitive ATPase activity and expressed as μmol Pi/mg protein/10 min. Protein in the homogenate was measured by the method of Lowry.[19] Mean activity in the injured spinal cord was calculated from the results determined for the two samples of each injured cord.

The activity of Na^+-K^+-ATPase was expressed as mean ± SD. Statistical analysis of the comparisons between placebo and TRH groups used the one-way unpaired t-test.

Results

The mean preinjury physiological parameters, MABP, heart rate, pH, PO_2, and PCO_2 were not statistically different ($p > 0.05$) between the two groups. Postinjury the MABP was significantly decreased ($p < 0.01$) and the heart rate significantly increased ($p < 0.01$) in both groups (Table 1).

The Na^+-K^+-ATPase activity in the injured spinal cord was 0.311 ± 0.053 μmol Pi/mg protein/10 min in the placebo group and 0.389 ± 0.073 μmol Pi/mg protein/10 min in the TRH group. This difference was statistically significant ($p = 0.024$).

Discussion

A variety of agents are effective in ameliorating spinal cord injury in experimental animals, including the synthetic glucocorticoid methylprednisolone sodium succinate, the antioxidants vitamin E, selenium, and dimethyl sulfoxide, the opioid antagonist naloxane, and TRH. Except for TRH, all of these agents have significant antioxidant and/or anti-lipid-hydrolysis properties.[2] Immunoreactive TRH and TRH receptors have been identified in the spinal cord.[6,20,24,25] Some studies used TRH in an experimental spinal cord injury.[1,3,4,6,7,14,16] Faden and Jacops[11] suggested that TRH analogs may be useful in spinal cord injury. TRH may serve as an excitatory neurotransmitter within the spinal cord.[10] TRH can penetrate the blood-brain barrier as determined by the measurement of spinal cord TRH immunoreactivity,[23] indicating the potential for a central site for the action of TRH. Some experimental studies have demonstrated that TRH administration produces recovery of evoked potentials after spinal cord injury.[5,6,15,22]

Cell membrane damage in the central nervous system following cerebral ischemia and spinal cord injury may be induced by free-radical reaction and lipid peroxidation.[9,18,19] The impact injury may result in a biomolecular injury to the axonal membrane.[17,18] The resting membrane potential is almost exclusively dependent on the relative intracellular and extracellular concentrations of potassium, which are maintained by the Na^+-K^+-dependent ATPase system (sodium pump).[18] The Na^+-K^+-ATPase is a plasma membrane-associated protein complex that is ex-

Table 1 Physiological parameters

Group	MABP (mmHg)	Heart rate (beat/min)	Parameters of arterial blood gas		
			pH	PO_2 (mmHg)	PCO_2 (mmHg)
Placebo (n = 8)					
preinjury	112.4 ± 3.4	472 ± 17	7.371 ± 0.03	85.7 ± 6.9	43.7 ± 4.3
1 hr postinjury	89.5 ± 4.7*	488 ± 29*	NM	NM	NM
2 hr postinjury	95.8 ± 6.2	432 ± 19	7.382 ± 0.04	82.6 ± 7.8	38.6 ± 9.1
TRH (n = 9)					
preinjury	108.4 ± 3.9	462 ± 12	7.381 ± 0.02	87.7 ± 6.2	44.1 ± 3.3
1 hr postinjury	92.9 ± 8.7*	498 ± 20*	NM	NM	NM
post-TRH treatment	98.8 ± 6.1	452 ± 16	7.384 ± 0.04	92.6 ± 7.2	39.6 ± 6.1

*$p < 0.01$ *vs.* preinjury. NM: not measured.

pressed in most eukaryotic cells.[21] Na^+-K^+-ATPase, which maintains the resting membrane potential physiologically, is a phospholipid-dependent membrane-bound enzyme.[19,26] The activity of this enzyme decreased in 5 minutes after spinal cord injury in dogs.[8,19] The improved phosphodiester/phosphomonoester ratio is correlated with improved adenosine triphosphate status after TRH treatment in spinal cord injury[12] and changes in tissue cations (Na^+, K^+, Mg^{2+}) and water content were reduced with TRH treatment.[1,12] In our investigation, the administration of TRH resulted in a higher level of Na^+-K^+-ATPase activity in the injured spinal cord. This greater activity may partly explain the changes in tissue cations and water content in spinal cord injury after TRH treatment, and also provides a biochemical basis for the beneficial effects of TRH in spinal cord injury.

Acknowledgment

This work was supported by the Black Sea Technical University Research Foundation.

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Address reprint requests to: S. Baykal, M.D., K.T.U. Lojmanları, 27/7, 61080, Trabzon–Turkey.