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Cholinergic receptor alterations in the cerebral cortex of spinal cord injured rat

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ABSTRACT

Many areas of the cerebral cortex process sensory information or coordinate motor output necessary for control of movement. Disturbances in cortical cholinergic system can affect locomotor coordination. Spinal cord injury causes severe motor impairment and disturbances in cholinergic signalling can aggravate the situation. Considering the impact of cortical cholinergic firing in locomotion, we focussed the study in understanding the cholinergic alterations in cerebral cortex during spinal cord injury. The gene expression of key enzymes in cholinergic pathway - acetylcholine esterase and choline acetyl transferase showed significant upregulation in the cerebral cortex of spinal cord injured group compared to control with the fold increase in expression of acetylcholine esterase prominently higher than cholineacetyl transferase. The decreased muscarinic receptor density and reduced immunostaining of muscarinic receptor subtypes along with down regulated gene expression of muscarinic M1 and M3 receptor subtypes accounts for dysfunction of metabotropic acetylcholine receptors in spinal cord injury group. Ionotropic acetylcholine receptor alterations were evident from the decreased gene expression of alpha 7 nicotinic receptors and reduced immunostaining of alpha 7 nicotinic receptors in confocal imaging. Our data pin points the disturbances in cortical cholinergic function due to spinal cord injury; which can augment the locomotor deficits. This can be taken into account while devising a proper therapeutic approach to manage spinal cord injury.

1. Introduction

Understanding the central nervous system pathways affected during spinal lesions as in the case of spinal cord injury will help to establish a proper therapy for patients with such devastating conditions. The changes at the level of neurotransmitters in the motor cortex during spinal cord injury are of special importance to understand the exact molecular mechanisms of brain- spinal cortex motor coordination. Many studies reported the involvement of various cortical regions in regulating the locomotor function during functional recovery in stroke patients [12,16,23,5]. Most of these studies are insufficient in concluding the exact firing mechanisms involved in this functional recovery and hence understanding the role of neurotransmitter alterations in cerebral cortex can shed light to its role in motor control.

ACh release from the frontal cortex and hippocampus has several components, one of which is motor activity. The involvement of cholinergic receptors, both muscarinic and nicotinic, in regulating spinal locomotor network is already known [19,20,25]. Central muscarinic receptors are known to play key roles in memory and learning as well as in the regulation of many sensory, motor, and autonomic processes [11]. It is reported that muscarinic cholinergic effects of ACh are important in the normal function of both the sensory and motor systems [7]. In mammals, nicotinic AChR also play a crucial role in motor control [15]. Following peripheral nerve injury, the expression of numerous receptors involved in nociceptive processing is altered in the superficial dorsal horn of the spinalcord. Activation of nAChR promotes survival of chicken spinal motoneurons that would otherwise undergo apoptosis when deprived of trophic factors [17]. Among the nicotinic acetylcholine receptors, alpha 7 subunit is reported to have an important role in motor control [25].

ACh is synthesized primarily by choline acetyltransferase (ChAT) from coenzyme A and choline [24]. In neurons, ACh is transported into vesicles by the vesicular ACh transporter, the entire coding sequence of which is contained in the first intron of the ChAT gene in mammals [2,6]. ACh is broken down by acetylcholinesterase (AChE), which is expressed in most tissues. Therefore ACh is confined to its area of synthesis and release and hence the quantification of its metabolic enzymes can be a direct index of Ach activity. Many previous studies

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used ChAT as a marker for the status of cholinergic transmission [10,21,9] and indicators of the functional stage of cholinergic neurons in the CNS [1]. Cholinergic input can be assessed by the activity of A ChE and ChAT [4,14].

The present study was designed to investigate the regulation of cholinergic function in the cerebral cortex of spinal cord injured rats. The cholinergic function was studied by evaluating the mRNA expression of its two key enzymes- AChE and ChAT. The receptor level changes were analyzed for metabotropic muscarinic receptors by receptor assays for total muscarinic, muscarinic M1 and M3 receptor subunits and gene expression studies using Real Time PCR. The ionotropic nicotinic receptors expression were also evaluated using Real Time PCR analysis and confocal imaging of alpha 7 nicotinic acetyl choline receptors using FITC tagged secondary antibodies. This study will help to open up new possibilities for a better therapy to deal with motor deficits in spinal cord injured patients.

2. Materials and methods

2.1. Animals

Male adult Wistar rats of 200–250 g body weight were used for all experiments. They were housed in separate cages under 12-h light and 12-h dark periods and were maintained on standard food pellets and water ad libitum. All animal care and procedures were in accordance with Institutional and National Institute of Health guidelines and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

2.2. Chemicals

Biochemicals used in the present study were purchased from Sigma Chemical Co., St. Louis, USA. All other reagents of analytical grade were purchased locally. Quinuclidinyl benzilate, L-[Benzilic-4, 4′-3H], ([3H] QNB) (Sp. Activity 42 Ci/mmol) and 4-DAMP, [N-methyl-3H] (Sp. Activity 83 Ci/mmol) were from NEN Life Sciences Products Inc., Boston, USA. Atropine, Pirenzepine and 4-DAMP were from Sigma Chemical Co., USA. Tri-reagent kit was purchased from MRC, USA. Real-time-PCR Taqman probe assays on demand were from Applied Biosystems, Foster City, CA, USA.

2.3. Induction of spinal cord injury and treatment group

Adult Wistar male rats (weight) were randomly divided into the following groups (a) Control (b) Spinal cord injured (SCI). Sham operated rats were used as control. Spinal cord injury was induced in adult Wistar rats by shearing between the T12 and T13 vertebra. The monoplegic rats were kept for 21 days and sacrificed by decapitation on the 22nd day of the experiment. The cerebral cortex was dissected out quickly over ice and the tissues were stored at −80 °C for various experiments.

2.4. Behavioural analysis of motor function by rotarod test

Rotarod has been used to evaluate motor coordination by testing the ability of rats to remain on revolving rod. The apparatus has a horizontal rough metal rod of 3 cm diameter attached to a motor with variable speed. This 70 cm long rod was divided into four sections by wooden partitions. The rod was placed at a height of 50 cm to discourage the animals to jump from the rotating rod. The rate of rotation was adjusted in such a manner that it allowed the normal rats to stay on it for five minutes. Each rat was given five trials before the actual reading was taken. The readings were taken at 10, 15 and 25 rpm after 21 days of treatment in all groups of rats.

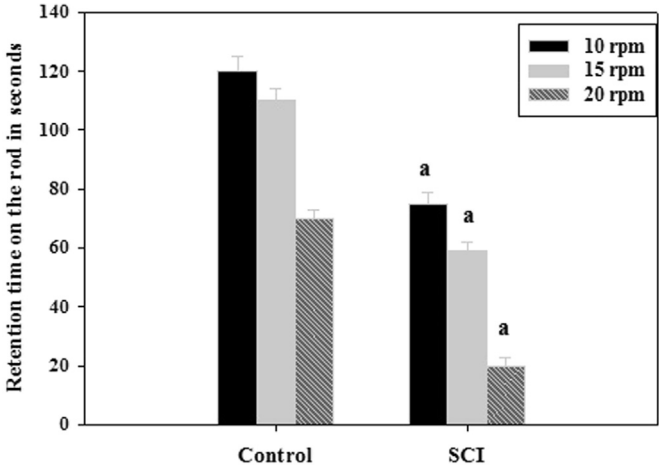

Fig. 1. Retention time of experimental rats in rotarod. Values are mean ± S.E.M of 4–6 separate experiments. Each group consists of 4–6 rats. [a] $p < 0.001$ when compared to control. C – Control, SCI – Spinal cord injury group.

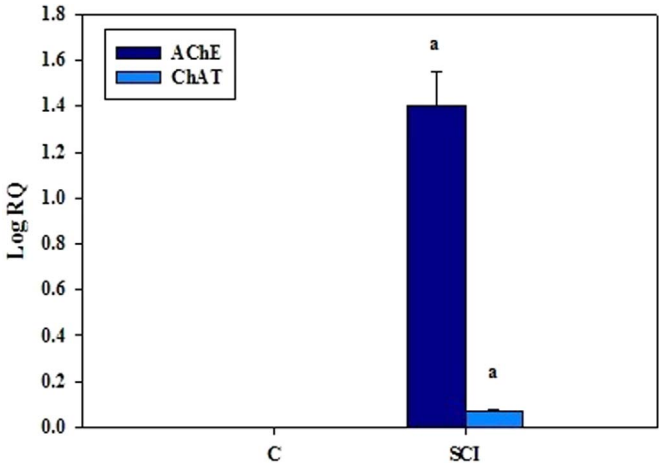

Fig. 2. Real Time PCR amplification of acetylcholine esterase and choline acetyl transferase mRNA in the cerebral cortex of control and experimental rats. Values are mean ± S.E.M of 4–6 separate experiments. Each group consists of 4–6 rats. [a] $p < 0.001$ when compared to control. C – Control, SCI – Spinal cord injury group.

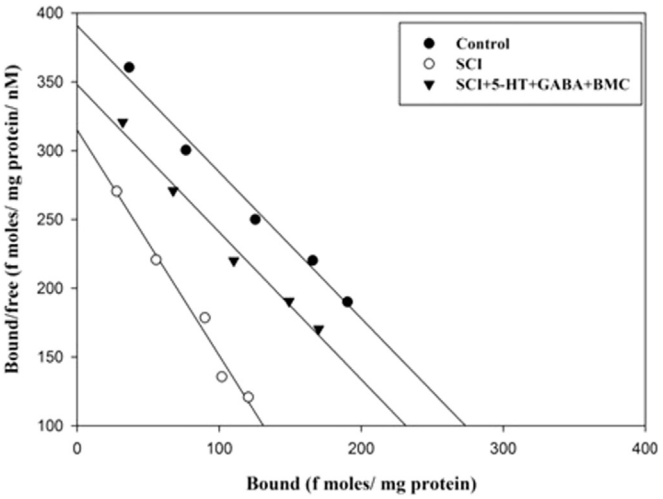

Fig. 3. Scatchard analysis of [^{3}H] QNB binding against atropine to total muscarinic receptor in the cerebral cortex. Values are mean ± S.E.M of 4–6 separate experiments. Each group consists of 4–6 rats. [a]$p < 0.001$ when compared to control. C – Control, SCI – Spinal cord injury group.