GABA<sub>A</sub> SUBUNIT GENE EXPRESSION IN THE BRAINSTEM OF TEMPORAL LOBE EPILEPTIC RATS: EFFECT OF Bacopa monnieri AND BACOSIDE–A

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ABSTRACT

Temporal Lobe Epilepsy (TLE) is thought to occur in 25% of children and approximately 50% of adults with known seizure disorders. Temporal lobe epilepsy seizures could originate in the medial or lateral neocortical temporal region, and many of these patients are refractory to medical treatment. GABA<sub>A</sub> is the principal inhibitory neurotransmitter in the mammalian brain involved in the pathophysiology of temporal lobe epilepsy. Treatment without having any influence on the course of the disease. Epilepsy is associated with behavioural deficits and many of the antiepileptic drugs also induce serious behavioural deficits. Thus, there is a pressing need to develop alternative therapeutic approaches that prevent the epileptogenesis. In the Indian medicinal system - Ayurveda, Bacopa monnieri has been used since 3000 years as a memory enhancing, antioxidative, adaptogenic, anti-inflammatory, analgesic, antipyretic, sedative and antiepileptic agent (Litt et al., 2001).

MATERIALS AND METHODS

Induction of Epilepsy

Epilepsy was induced in Adult Wistar rats of 250-300g body weight, by injecting with pilocarpine (350 mg/kg body weight), preceded by 30 min with atropine (1 mg/kg body weight) to reduce peripheral pilocarpine effects. Within 20 to 40 minutes after the pilocarpine injection, essentially all the animals developed status epilepticus. Control animals were given saline injection. Experimental rats were divided into five groups: 1) Control, 2) Epileptic, 3) Epileptic rats treated with Bacopa monnieri, 4) Epileptic rats treated with bacoside-A, 5) Epileptic rats treated with carbamazepine. Bacopa monnieri treated rats were given extract of Bacopa monnieri orally in the dosage 300mg/kg body weight/day for 15 days. Carbamazepine, a standard drug used for the treatment of epilepsy was given orally in the dosage 150mg/kg body weight/day for 15 days. Bacoside-A was given orally in the dosage 150mg/kg body weight/day for 15 days.

Isolation of RNA

Tissue (25-50 mg) homogenates were made in 0.5 ml Tri Reagent and was centrifuged at 12,000xg for 10 minutes at 4°C. The clear supernatant was transferred to a fresh tube and it was allowed to stand at room temperature for 5 minutes. 100μl of chloroform was added to it, mixed vigorously for 15 seconds and allowed to stand at room temperature for 15 minutes. The tubes were then centrifuged at 12,000xg for 15 minutes at 4°C. Three distinct phases appear after centrifugation. The bottom red organic phase contained protein, interphase contained DNA and a colourless upper aqueous phase contained...
RNA. The upper aqueous phase was transferred to a fresh tube and 250 µl of isopropanol was added and the tubes were allowed to stand at room temperature for 10 minutes. The tubes were centrifuged at 12,000xg for 10 min at 4°C. RNA precipitate forms a pellet on the sides and bottom of the tube. The supernatants were removed and the RNA pellet was washed with 500 µl of 75% ethanol, vortexed and centrifuged at 12,000xg for 5 min at 4°C. The pellets were semi dried and dissolved in minimum volume of DEPC-treated water. 2 µl of RNA was made up to 1 ml and absorbance was measured at 260nm and 280nm in spectrophotometer (Shimadzu UV-1700 pharma SPEC). For pure RNA preparation the ratio of absorbance at 260/280 was 2. The concentration of RNA was calculated as one absorbance 260 = 40 µg.

cDNA Synthesis

Total cDNA synthesis was performed using ABI PRISM cDNA Archive kit in 0.2ml microfuge tubes. The reaction mixture of 20 µl contained 0.2 µg total RNA, 10X RT buffer, 25X dNTP mixture, 10X Random primers, MultiScribe RT (50U/µl) and RNase free water. The cDNA synthesis reactions were carried out at 25°C for 10 minutes and 37°C for 2 hours using an Eppendorf Personal Cycler. The primers and probes were purchased from Applied Biosystems, Foster City, CA, USA designed using Primer Express Software Version (3.0).

Real-Time PCR Assay

Real Time PCR assays were performed in 96-well plates in a ABI 7300 Real Time PCR instrument (Applied Biosystems). To detect gene expression, a TaqMan quantitative 2-step reverse transcriptase polymerase chain reaction (RT-PCR) was used to quantify mRNA levels. First-strand cDNA was synthesized with use of a TaqMan RT reagent kit, as recommended by the manufacturer. PCR analyses were conducted with gene-specific primers and fluorescently labelled TaqMan probe, designed by Applied Biosystems. Endogenous control, β-actin, was labelled with a reporter dye (VIC). All reagents were purchased from Applied Biosystems. The probes for specific gene of interest were labeled with FAM at the 5’ end and a quencher (Minor Groove Binding Protein - MGB) at the 3’ end. The Real-Time data was analyzed with Sequence Detection Systems software version 1.7. All reactions were performed in duplicate.

RESULTS

Figure 1: Real Time PCR amplification of GABA_Aα1 receptor subunit mRNA from the brain stem of control and experimental rats (C-Control, E-Epileptic, E+B-Epileptic rats treated with *Bacopamonnieri*, E+D-Epileptic rats treated with *Bacoside A* and E+C-Epileptic rats treated with Carbamazepine)
DISCUSSION

Neurological functions located in the brainstem include those necessary for survival. The brainstem is the pathway for all fiber tracts passing up and down from peripheral nerves and spinal cord to the highest parts of the brain. GABA is a major inhibitory neurotransmitter in the mammalian brain stem. In our study GABA content was significantly decreased in the brain stem of the epileptic rats compared to control. Real Time PCR
amplification of GABA<sub>A</sub> receptor subunits such as GABA<sub>Aδ</sub>, GABA<sub>Aγ</sub> and GABA<sub>Aδ</sub> were down regulated in the brain stem of the epileptic rats compared to control. Bacopa monnieri and Bacoside A treatment reversed these changes near to control.

Synaptically released GABA activates postsynaptic GABA<sub>A</sub> receptors, which increase the membrane permeability to chloride, evoking a hyperpolarizing inhibitory postsynaptic current (IPSC). Synaptic release increases the concentration of GABA to a relatively high level (millimolar range) within the synapse (Farrant & Nusser, 2005). The brief current evoked by synaptic release of GABA from presynaptic terminals is referred to as "phasic" inhibition. In monkeys made epileptic by cortical application of alumina gel, a highly significant numerical decrease of GAD-positive nerve terminals occurred at sites of seizure foci indicating a functional loss of GABAergic inhibitory synapses (Ribak et al., 1979). Loss of such inhibition at seizure foci could lead to epileptic activity of cortical pyramidal neurons. Long-term GABA<sub>A</sub> receptor alterations occur in hippocampal dentate granule neurons of rats that develop epilepsy after status epilepticus in adulthood (Zhang et al., 2004). Abnormalities of GABAergic function have been observed in genetic and acquired animal models of epilepsy. Reductions of GABA mediated inhibition and decreased activity of glutamate decarboxylase has been reported in studies of human epileptic brain tissue. Our study showed that GABA receptors were decreased in the brain stem of the epileptic rats and treatment using Bacopa monnieri and Bacoside A increased the GABA receptor density providing an antiepileptic effect.

The mechanism of action behind the memory and cognition enhancing effects of Bacopa monnieri is still uncertain, as its multiple active constituents have multifunctional properties, making its pharmacology complex. But the antioxidant properties of Bacopa monnieri have been well documented (Brusa et al., 1995). Glutamate is one of the chief excitatory amino acids that mediate excitotoxic neuronal degeneration. Treatment with Bacopa monnieri extract reduced the increase in glutamate dehydrogenase activity to near-control levels. Hence, it is suggested that Bacopa monnieri has a definite role in decreasing glutamate excitotoxicity. Bacopa monnieri treatment induce membrane dephosphorylation and a concomitant increase in mRNA turnover and protein synthesis. It can also enhance protein kinase activity in the hippocampus, which is critically involved in learning and memory (Sumathy et al., 2002). The water maze experiment conducted by Reas et al. (2007) to study the neurobiological mechanisms that underlie spatial learning and memory function in epileptic rats. The hippocampal formation is critical for computing place representations. The Morris water maze experiment demonstrated the impairment in spatial learning during epilepsy. Escape latency was increased in epileptic rats as compared with control rats. Treatment using Bacopa monnieri and Bacoside A increase the performance in Morris water maze (Reas et al., 2008).

Thus our results support that decreased GABA receptors and GAD activity in the brain stem comprise an important role in the TLE seizures. We concluded that Bacopa monnieri and Bacoside A treatment giving a beneficial effect by reversing the alterations in general GABA, GABA<sub>Aδ</sub>, GABA<sub>Bδ</sub> receptor binding, GABA<sub>A</sub> receptor subunits and GAD gene expression.

CONCLUSION

GABAergic system has an important regulatory role in the pathophysiology of pilocarpine induced temporal lobe epilepsy in rats. We observed a decreased GABA subunit gene expression in the brain stem of the epileptic rats which showed the deficit in GABA mediated inhibition. Decreased GABA receptor functional regulation leads to seizures in epileptic rats. Epileptic rats show impairment in the behavioral tests, for assessing the motor learning and memory. Treatment using Bacopa monnieri and bacoside A decreased the seizure frequency in the epileptic rats. Bacopa monnieri and bacoside A upregulated GABA<sub>A</sub> subunit gene expression. Increased GABA receptors in the brain regions are suggested to prevent the occurrence of seizures in epileptic rats.

REFERENCES


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