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BRAIN RESEARCH

Research Report

Vagus nerve stimulation may protect GABAergic neurons following traumatic brain injury in rats: An immunocytochemical study

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ABSTRACT

Seizures and subclinical seizures occur following experimental brain injury in rats and may result from inhibitory neuron loss. This study numerically compares cortical and hippocampal glutamic acid decarboxylase (GAD) positive neurons between sham fluid percussion injury (FPI), FPI with sham Vagus Nerve Simulation (VNS), and FPI with chronic intermittent VNS initiated at 24 h post FPI in rats. Rats (n=8/group) were prepared for immunocytochemistry of GAD at 15 days post FPI. Serial sections were collected and GAD immunoreactive neurons were counted in the hippocampal hilus and two levels of the cerebral cortex. Numbers of quantifiable GAD cells in the rostral cerebral cortices were different between groups, both ipsilateral and contralateral to the FPI. Post hoc analysis of cell counts rostral to the ipsilateral epicenter, revealed a significant 26% reduction in the number of GAD cells/unit area of cerebral cortex following FPI. In the FPI-VNS group, this percentage loss was attenuated to only an 8.5% reduction, a value not significantly different from the sham group. In the contralateral side of the rostral cerebral cortex, FPI induced a significant 24% reduction in GAD cells/unit area; whereas, the VNS-treated rats showed no appreciable diminution of GAD cells rostral to the contralateral epicenter. Hippocampal analysis revealed a similar reduction of GAD cells in the FPI group; however, unlike the cortex this was not statistically significant. In the FPI-VNS group, a trend towards increased numbers of hilar GAD cells was observed, even over and above that of the sham FPI group; however, this was also not statistically significant. Together, these data suggest that VNS protects cortical GAD cells from death subsequent to FPI and may increase GAD cell counts in the hippocampal hilus of the injured brain.

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1. Introduction

Our recent studies demonstrate that vagus nerve stimulation (VNS), a therapeutic approach to seizure control in humans (Schachter and Wheless, 2002; review), markedly facilitates the recovery of locomotor and cognitive function in rats that have been subjected to unilateral fluid percussion injury (FPI) (Smith et al., 2005, 2006). The mechanism of this facilitation remains an ardent focus of our studies and we have hypothesized that interactions between gamma aminobutyric acid (GABA), norepinephrine (NE), and VNS may be involved in functional recovery and seizure suppression (Smith et al., 2005, 2006).

Seizure disorders often develop in the weeks following traumatic brain injury in humans (Frey, 2003; Pitkanen and McIntosh, 2006) and may exacerbate associated morbidity (Asikainen et al., 1998). Seizure activities, including subclinical seizures verified with electrophysiology, also occur following experimental brain injury in rats (Santhakumar et al., 2001; D'Ambrosio et al., 2004). Seizure activity has been recorded at 1 and 2 weeks following fluid percussion injury (FPI) with seizure foci first detected in the cerebral cortices adjacent to the injury site. However, by 27–28 weeks post-injury, seizure activities manifest as hippocampus-initiated focal seizures (D'Ambrosio et al., 2005).

Data from previous studies suggest that the seizure suppressive effects of VNS may be partially subserved by the locus coeruleus (LC; Krahl et al., 1998), a pontine nucleus that produces the majority of NE in the brain. We, and others, have helped establish that NE is a primary determinant of seizure severity in both genetic as well as non-genetic seizure models (Kilian and Frey, 1973; Maynert et al., 1975; Buterbaugh and London, 1977; Jobe et al., 1986; Browning, 1987; Clough et al., 1994; Weinshenker and Szot, 2002). Generally, reductions in NE promote increased severity of seizures whereas elevated NE can suppress seizure activity. Moreover, an antiepileptogenic role of NE has been demonstrated in models of epileptogenesis including electrogenic kindling (McIntyre, 1980). A now classic study by Naritoku et al. (1995) showed that VNS causes an increase in fos labeling in the LC, presumably as a result of intense neuronal activation. We have shown a similarly increased activation in the LC as a consequence of a mixed variety of seizure evoking stimuli (Eells et al., 1997). Finally, our recent microdialysis/HPLC studies demonstrate that VNS increases NE release within the hippocampal formation and the cerebral cortex (Roosevelt et al., in press). Thus, hypothetically, VNS may promote recovery of function via seizure suppressive effects in part through activation of NE release into the brain. This hypothesis remains under investigation.

Certain aspects of brain pathology also appear to correlate with seizure development subsequent to experimental FPI. For example, glial fibrillary acidic protein (GFAP) staining is prominent at 2–3 weeks post FPI in the ipsilateral cerebral cortex; whereas, at 27–28 weeks following injury, significant GFAP elaboration is observed in the temporal lobe compared to a 2–3 week time point (D'Ambrosio et al., 2005). Additionally, there is no apparent hippocampal shrinkage at 2–3 weeks after FPI; however, varying degrees of hippocampal and temporal lobe asymmetry are present at 27–28 weeks. Structural changes, thought to be related to increasing seizure propensity, are also apparent in mossy fiber systems of the hippocampal dentate gyrus subsequent to FPI (Santhakumar et al., 2001). Furthermore, FPI is associated with a loss of up to 50% of hilar neurons in the ipsilateral hippocampus and a pronounced period of hyper-excitability in hippocampal pyramidal neurons (Lowenstein et al., 1992; Toth et al., 1997; Santhakumar et al., 2001). We and others have shown a significant loss of CA3–CA1 pyramidal neurons over 2 weeks following experimental FPI in rats (Smith et al., 2005; Anderson et al., 2005) that apparently coincides with the temporal profile of excitability (Santhakumar et al., 2001). Continued loss of pyramidal cells in the Cornu Amonis of the human hippocampus after brain injury has also been described (Kotopka et al., 1992; Maxwell et al., 2003). Cortical neuron loss, as may be expected, both in and around the epicenter of experimental brain injury has been widely described (McIntosh et al., 1989; Smith et al., 1997). These events, particularly the loss of cortical neurons and derangement of their circuitry, loss of hilar neurons (including, presumably, inhibitory GABA neurons), mossy fiber sprouting, and resulting hyper excitability of the pyramidal cells, appear to set the stage for predisposition to seizures. Thus, the development of post-traumatic seizures that occurs following brain injury may involve a loss of GABAergic cells and inhibitory tone in the brain.

A role for the inhibitory neurotransmitter GABA in VNS-mediated seizure suppression in humans undergoing VNS therapy is also suggested. At both high and low amplitude VNS, total GABA levels in the cerebrospinal fluid of VNS patients are significantly increased (Ben-Menachem et al., 1995). Additionally, single photon emission computed tomography (SPECT) of $GABA_A$ receptor density in the hippocampus following 1-year of VNS therapy showed a significant normalization of GABA-receptor density that correlated with seizure reduction (Marrosu et al., 2003). Regulation of GABAergic signaling involves the synthesis of GABA from glutamate by the enzyme glutamic acid decarboxylase (GAD). Two isoforms of GAD exist: GAD_{65}, which is predominantly located in membranes and axon terminals, and GAD_{67}, which is distributed widely in cells (Soghomoninan and Martin, 1998). The majority of GABAergic cells express both isoforms and thus either or both forms of GAD can be used with immunocytochemistry to label GABAeric neurons. Insofar as VNS is associated with an increased steady state GABA level in humans being treated for epilepsy, we hypothesize that the VNS may have a protective effect on GAD neurons in the cerebral cortex as well as the hippocampus that may otherwise be jeopardized in traumatic brain injury.

2. Results

2.1. *GAD immunohistochemistry*

Fig. 1 depicts representative photomicrographs of $GAD_{65/67}$-like immunopositive cells within the cerebral cortex (panels A and C) and within the hippocampus (panels B and D). The areas used for counting the $GAD_{65/67}$-like cells in the cerebral cortices and the hippocampal hilar region are graphically

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