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Research Report

Post-hypoxic myoclonus induces Fos expression in the reticular thalamic nucleus and neurons in the brainstem

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Abstract

Post-hypoxic myoclonus is a movement disorder characterized by brief, sudden involuntary muscle jerks. Although the mechanism underlying this disorder remains unclear, earlier pharmacological studies indicated that aberrant activity of specific neuronal circuitry in the central nervous system causes this disorder. In the present study, Fos protein, an immediate-early gene product, was used as a marker of neuronal activity to identify the brain nuclei possibly involved in post-hypoxic myoclonus. We found that Fos protein was immunologically detected in the reticular thalamic nucleus (RT), the medial longitudinal fasciculus (MLF) as well as in the locus coeruleus (LC) and the periventricular gray substance (PVG) in post-hypoxic rats that developed myoclonus in response to auditory stimuli. Fos was not detected in these nuclei from rats that underwent 4 min of cardiac arrest without myoclonus. Electrolytic lesions of the RT or MLF but not the LC/PVG significantly reduced auditory stimulated myoclonus in the post-hypoxic rats. The results suggest that neuronal activity in the RT and the MLF plays a contributing role in post-hypoxic myoclonus.

Theme: Disorders of the nervous system
Topic: Ischemia

Keywords: Post-hypoxic myoclonus; Fos; Immediate-early gene; Reticular thalamic nucleus; Medial longitudinal fasciculus; Locus coeruleus; Periventricular gray substance; Electrolytic lesion

1. Introduction

Post-hypoxic myoclonus is neurological disorder characterized by brief, sudden, shock-like involuntary movements caused by active muscle contractions or inhibitions [9]. Cerebral ischemic/hypoxic insults during cardiac arrest are one of the main causes of post-hypoxic myoclonus. Although the mechanism underlying post-hypoxic myoclonus remains obscure, the response of this condition to certain pharmacological agents provides implications on the mechanism underlying this disorder. For example, administration of classical anti-epileptic agents such as clonazapam [27] and valproate [4,18,28] in patients with post-hypoxic myoclonus improves their myoclonus symptoms. The principal action of these anti-epileptic agents is to enhance the inhibitory GABAergic neurotransmission of the neural circuitries in the brain by increasing the GABA receptor activity. Furthermore, in animal studies, activation of GABA receptors has anti-myoclonic effects, whereas blockade of the GABA receptors by intraventricular infusion of $GABA_A$ receptor antagonists such as bicuculline elicits myoclonus in a dose-related manner [14]. These results support the notion that post-hypoxic myoclonus is the result of hyperactivity of specific neural circuits in the brain whose activities are substantially elevated following cerebral hypoxic injury. Identification of these brain nuclei will, therefore, provide new insights into the mechanism of this disorder.

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Since the early 90s, neuronal activity is known to cause a rapid induction of a class of proteins coded for by immediate-early genes such as c-fos [12]. Fos protein is synthesized during certain forms of neuronal activation as a result of intraneuronal metabolic changes. This unique feature of c-fos expression enables it to be a valuable marker to identify the neuronal cell groups that are activated in response to exogenous stimuli. It has been shown that neuronal activity whether it is triggered electrically [1,3,23] or chemically such as administration of chemical convulsants [11,13] induces a rapid and transient increase of c-fos expression in rat brain. In the present study, Fos protein was used a functional marker for neuronal activation to identify hyperactive brain nuclei in an established animal model of post-hypoxic myoclonus [7,8,24,25]. Using this approach, we have identified discrete nuclei in the thalamus and the brainstem whose hyperactivity following cerebral hypoxic insult may cause post-hypoxic myoclonus.

2. Methods and experimental procedures

2.1. Animal model of cardiac arrest-induced post-hypoxic myoclonus

An animal model of post-hypoxic myoclonus originally developed by Truong et al. [24] was used in this study. In brief, Sprague–Dawley rats of 220–240 g were anesthetized with ketamine (85 mg/kg, i.p.) and xylazine (15 mg/kg, i.p.). Atropine (0.04 mg/kg, i.p.) was administrated to minimize respiratory secretion. The trachea was intubated with an 18-gauge catheter, which was then attached to a ventilator (settings: 425 ml/min; 60 strokes/min). The rat was placed on a heating pad, and ECG electrodes were attached. Body temperature was maintained at 37 °C with a heating lamp. The left femoral artery and vein were catheterized to monitor arterial blood pressure and for administration of drugs, respectively. An incision of an inch length was made on the skin along the rat cavity at a location above the heart. The skin was pulled apart to facilitate the insertion of an L-shaped metal loop into the rat cavity. An L-shaped metal loop was inserted through the muscle of the rat body cavity into an area underneath the aorta and the surrounding major blood vessels. Cardiac arrest was initiated and maintained by mechanically obstructing all the major cardiac blood vessels including the aorta by applying pressure on the surface of the rat cavity with the palm of one hand while pulling the inserted L-shaped loop up against the rat cavity with another hand. The arterial blood pressure was maintained at 0–10 mm Hg. Under such low systemic arterial blood pressure, cerebral perfusion came to a halt. Post-hypoxic rats developed myoclonus only after a critical duration of cerebral hypoxia. A cardiac arrest for a duration of 9 min 30 s is a compromise at which myoclonus reliably develops following recovery, the survival rate is acceptable, and the severity of other neurological conditions was manageable.

Resuscitation began at 9 min 30 s following cardiac arrest by resuming manual thoracic compression and by intravenous injection of 10 mg/kg epinephrine and 4 mEq/kg sodium bicarbonate. Following resuscitation, rats were weaned from the ventilator, the catheters were removed, and wounds were sutured. The animals were placed on a heating pad to facilitate recovery from surgical coma.

2.2. Behavior and evaluation of post-hypoxic myoclonus in rat

One of the clinical features of post-hypoxic myoclonus is the trigger of myoclonic jerks in response to exogenous stimuli such as sound. Jerking movements in response to auditory stimuli were ranked according to their intensity as previously described [24].

For the Fos staining experiments, 2 days after cardiac arrest, rats were given auditory stimuli. For the purpose of evaluating myoclonic jerks in response to auditory stimuli, the rat was placed in a clear plastic cage for 10 min to let it habituate to the new environment prior to evaluation. The rat was then presented with 45 clicks of a metronome as the auditory stimulus. Each click had a sound intensity of 96 dB with a duration of 40 ms at a frequency of 0.75 Hz. The involuntary muscle jerks in response to each click were scored with the following criteria: 0 = no jerks; 1 = ear twitch; 2 = ear and head jerk; 3 = ear, head and shoulder jerk; 4 = whole body jerk; and 5 = whole body jerk with jumping [7,24]. The myoclonus scores shown in Figs. 1 and 5 are the sum of the 45 scores of the rats in response to the 45 clicks. One hour after auditory stimulus, rats were sacrificed. Brain sections were prepared for detection of Fos protein as described below. Rats that underwent cardiac arrest for 4 min did not develop myoclonus when given auditory stimuli. This group would serve as a negative control for the study.

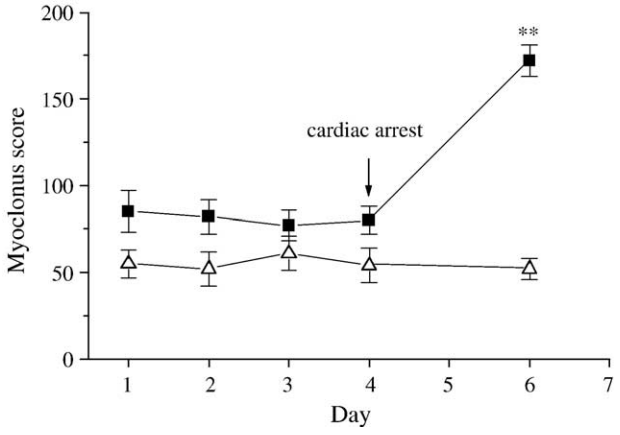

Fig. 1. Rats subjected to cardiac arrest for 9 min 30 s but not for 4 min developed myoclonus in response to auditory stimuli. Myoclonus scores recorded from rats before and following cardiac arrest for 9 min 30 s (■) or 4 min (Δ). The arrow indicates the day on which cardiac arrest surgery was performed. Values are mean ± SE. **Indicates significant difference from control at $P < 0.01$. Five animals were used in each group.