Pergamon

Pharmacology Biochemistry and Behavior, Vol. 52, No. 2, pp. 443–446, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00148-4

Regulation of Tyrosine Protein Kinase Receptor Trk-B and Motor Function in Rats Following Cardiac Arrest

S. P. JAW,[1] D. D. SU, Q.-V. T. VUONG AND D. D. TRUONG

Parkinson and Movement Disorders Laboratory, Department of Neurology, University of California Irvine, 154 Med Surge I, Irvine, CA 92717

Received 14 September 1994

JAW, S. P., D. D. SU, Q.-V. T. VUONG AND D. D. TRUONG. *Regulation of tyrosine protein kinase receptor Trk-B and motor function in rats following cardiac arrest.* PHARMACOL BIOCHEM BEHAV **52**(2) 443–446, 1995.– Following 10 min cardiac arrest and resuscitation, male Sprague–Dawley rats developed posthypoxic myoclonus. Sixty days later, the motor function of the animals was restored. In the present study, we investigated brain levels of tyrosine protein kinase receptor Trk-B with quantitative immunoblot analysis at various time points following cardiac arrest. In the frontal cortex, a significant reduction of Trk-B was found in rats 3 days (53%) after cardiac arrest, whereas significant increases were detected in rats 14 (124%) and an average 60 days (98%) after cardiac arrest. In the striatum, significant increases were found in rats 3 (389%), 14 (483%), and 60 days (521%) after resuscitation. In contrast, significant reductions of Trk-B were detected in the cerebellum of rats 3 (46%), 14 (22%), and 60 days (18%) after cardiac arrest. The results indicate that regulation of Trk-B may vary in different brain regions and have important roles in recovery processes following hypoxic-ischemic insults to the brain.

Hypoxia Ischemia Protein kinase receptors Trk-B Posthypoxic myoclonus

THE BRAIN-DERIVED neurotrophic factor (BDNF) and neurotrophin-4/5 (NT-4/5) are target-derived, retrogradely transported neurotrophic factors (9). They are widely distributed in the brain including areas related to motor function (9). Tyrosine protein kinase receptors Trk-B are high-affinity receptors for both BDNF and NT-4/5 (9). BDNF and NT-4/5 are known to have important roles in the development (8,13, 14,18), maintenance (3,4,15), and regeneration of the nervous system (1,9,10,16). However, regulations of BDNF and NT-4/5 receptors, Trk-B, in different pathophysiologic states remain elusive. Rats developed posthypoxic stimulus-sensitive myoclonus (involuntary contraction or inhibition of contraction of muscle groups) following 10-min cardiac arrest and resuscitation (6,17). This phenomenon peaked at 14 days and disappeared by 60 days after cardiac arrest (6,17). The mechanisms for the restitution of motor function in posthypoxic rats are not known. It is hypothesized that some processes in the developing brain may be reactivated in an adult brain following hypoxia-ischemia injuries and contribute to restoration of motor function in our model. We are interested in the effects of hypoxia-ischemia on Trk-B and roles of Trk-B in the recovery of motor function in posthypoxic rats. In the present studies, brain levels of Trk-B at various time points after cardiac arrest were therefore examined with quantitative immunoblot analysis. In addition, damage in the frontal cortex, striatum, and cerebellum was detected in the model (6,7). These areas of the brain either initiate or modulate motor function and are thought to be important in mediating myoclonus (6,7). Therefore, the frontal cortex, striatum, and cerebellum were chosen for the studies.

METHODS

Animals

We used 4- to 5-week-old male Sprague–Dawley rats (225–250 g; Zivic Miller, Zelinople, PA). They were maintained for 1 week before surgery on a 12 L : 12 D cycle (lights on 0600 h) and allowed food and water ad lib. All procedures were approved by the University of California Irvine Animal Care and Use Committee (IACUC).

[1] To whom requests for reprints should be addressed.

Cardiac Arrest and Resuscitation Procedures

We used the procedure for cardiac arrest described by Truong et al. (17). Briefly, before surgery, rats were fasted for at least 12 h. Animals were anesthetized with ketamine (100 mg/kg) and atropine (0.4 mg/kg), tracheotomized, intubated, and connected to a ventilator (Harvard Rodent Ventilator Model 683; South Natick, MA) with the following settings: 425 cc/min NO_2, 175 cc/min O_2, 60 strokes/min, 5 cm H_2O positive end-expiratory pressure (PEEP). A femoral artery and vein were catheterized for the measurement of blood pressure and the administration of drugs, respectively. Electrocardiogram and blood pressure were continuously recorded. Succinylcholine (2 mg/kg, IV) was used to paralyze the muscles of the animals and facilitate cardiac arrest, which was induced via transthoracic intracardial injections of ice-cold KCl (1%, 0.4 ml) and turning off the ventilator. Resuscitation was started 10 min after the arrest by turning on the ventilator (100% O_2, 100 strokes/min), manual compression of the animal chest, and IV injections of epinephrine (10 mg/kg) and sodium bicarbonate (4 mEq/kg). Rats were gradually weaned from the ventilator over 2–4 h; their wounds were sutured and the catheters were removed.

Behavioral Assessment

Rats were presented with a series of 45 clicks from a metronome (1 Hz, 95 dB, 40 ms), and the response to each click was scored as follows: 0 = no response; 1 = ear twitch; 2 = ear and head jerk; 3 = ear, head, and shoulder jerk; 4 = whole body jerk; and 5 = whole body jerk of such severity that it caused a jump. The total myoclonus score for each rat was determined by summing the scores yielded over 45 clicks.

Brain Homogenation

Control rats (n = 6) underwent the same procedures except for cardiac arrest. At various time points (3, 14, and 60 days) (n = 6 for each group) after resuscitation, behavioral scores of rats were assessed before sacrifice. The frontal cortex, striatum, and cerebellum were immediately removed, homogenised with 10 vol of 10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5 (buffer A), and centrifuged at 500 × g for 10 min. The supernatant was centrifuged further at 48,000 × g for 10 min, and pellets from the second centrifugation were washed with buffer A and recentrifuged at 48,000 × g for 10 min. Finally, pellets were resuspended in buffer A at a protein concentration of 4 mg/ml and stored at −80°C. Protein concentration was measured according to the method of Lowry et al. (11).

Immunoblotting

Rat brain membranes were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 14 × 16-cm slab gels. After resolution in 10% (wt./vol.) acrylamide SDS-PAGE, proteins were transferred to nitrocellulose (Schleicher and Schuell, Keene, NH) and blocked for 2 h at 37°C with 5% gelatin in Tris-buffered saline (TBS; 20 mM Tris-HCl, pH 7.5, 500 mM NaCl). Primary antibody (1 : 1000 dilution, antibodies against carboxy termini of Trk-B gp145; Santa Cruz Biotechnology, Santa Cruz, CA) in 1% gelatin-TBS was then added and left overnight. The primary antibody was then removed, and the blot was washed extensively with distilled water followed by washes with TBS containing 0.1% (vol./vol.) Tween 20 and then TBS. Secondary antibody (1 : 1000 dilution, goat anti-rabbit immunoglobulin G (IgG) coupled to horseradish peroxidase (BioRad, Hercules, CA) in 1% gelatin-TBS was added and left for 3 h. Removal of the secondary antibody was followed by the same series of extensive washes of the nitrocellulose membrane as detailed following removal of the primary antibody. 4-Chloro-1-naphthol (BioRad) was employed as the substrate for detection of the antibody complex.

Image Analysis

The developed immunoblots were scanned with a Micro-Computer Image Device (MCID; Imaging Research, Inc., Ontario, Canada) densitometer. The background was subtracted by scanning equivalent-size areas of nitrocellulose that did not contain immunoreactive protein.

Statistics

Changes in myoclonus scores and immunoreactivities were analyzed by one-way analyses of variance (ANOVA), followed by Dunnett's t-tests. $p < 0.05$ was considered to be significant.

RESULTS

Time Dependency of Expression of Posthypoxic Myoclonus

Rats developed posthypoxic myoclonus at 3 and 14 days following 10-min cardiac arrest and resuscitation (Table 1). However, this phenomenon disappeared after 60 days (Table 1).

Immunoblot Analysis of Trk-B in the Rat Brain

No significant changes in immunoreactivities of Trk-B were found between naive rats (no treatment) and those that received treatments except cardiac arrest. In the frontal cortex, a significant reduction of Trk-B immunoreactivities was found in rats 3 days (n = 6, 53%), whereas significant increases were detected in rats 14 (n = 6, 124%) and an average of 60 days (n = 6, 98%) after cardiac arrest [$F(3, 18) = 3.31$, $p < 0.05$] (Fig. 1). In the striatum, significant increases were found in rats 3 (n = 6, 389%), 14 (n = 6, 483%), and 60 days (n = 6, 521%) after resuscitation [$F(3, 18) = 3.78$, $p < 0.05$] (Fig. 1). In contrast, significant reductions of Trk-B were detected in the cerebellum of rats 3 (n = 6, 46%), 14 (n = 6, 22%), and 60 days (n = 6, 18%) after resuscitation [$F(3, 18) = 3.43$, $p < 0.05$] (Fig. 1).

DISCUSSION

After cardiac arrest and resuscitation, there were increases in immunoreactivities of Trk-B in the frontal cortex and stria-

TABLE 1

MYOCLONUS SCORES OF RATS BEFORE AND AT DIFFERENT DAYS AFTER 10 MIN CARDIAC ARREST AND RESUSCITATION

Basal	3 days	14 days	60 days
52 ± 6	178 ± 7**	184 ± 8*	54 ± 10

The data are expressed as means ± SEM Statistical significance was determined by ANOVA and posthoc Dunnett's t-test between basal values assessed from rats before cardiac arrest and those at different days after resuscitation.

*$p < 0.01$.

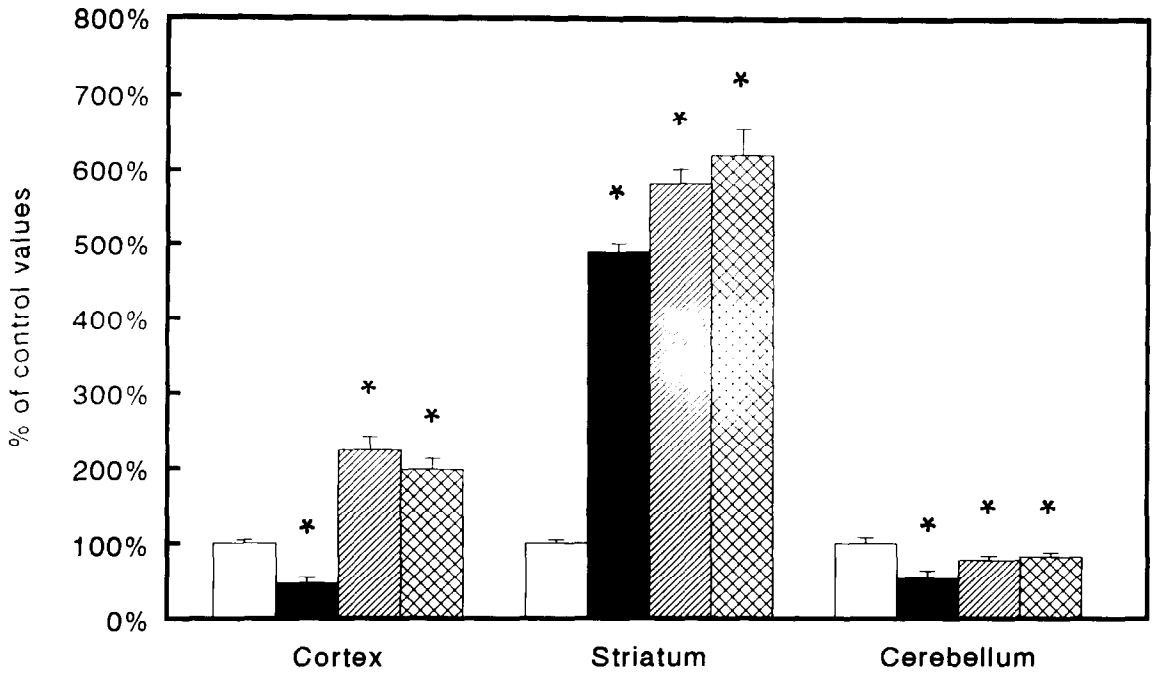

FIG. 1. Densitometric levels of Trk-B immunoreactivites (percent of control values) in the cerebral cortex, striatum, and cerebellum of rats: control (n = 6, open columns), 3 (n = 6, black columns), 14 (n = 6, striped columns), and an average of 60 days (n = 6, hatched columns) following cardiac arrest. The experiments were repeated at least three times from six rats. *$p < 0.05$; values significantly different from those of the control group as determined by ANOVA followed by Dunnett's t-test.

tum of rats. The results suggest that a greater number of receptors emerged or "uncovered" in posthypoxic rats. This phenomenon may be due to increased synthesis and/or decreased internalization and degradation of Trk-B in response to neuronal loss and/or degeneration following hypoxic-ischemic insults to the brain. On the other hand, there was a significant reduction in the immunoreactivities of Trk-B in the cerebral cortex (3 days) and cerebellum of posthypoxic rats. The results indicate that these brain regions were all affected by cardiac arrest, and the response in regulation of Trk-B differed in various brain regions (10,16).

Increases in the immunoreactivities of Trk-B in the frontal cortex and striatum took place following cardiac arrest. The results indicate that there may have been an increase in the number of functional Trk-B and/or truncated Trk-B in these areas of the rat brain. The truncated form of Trk-B lacking intracellular kinase domains is expressed at very high levels in the CNS and localized predominantly to nonneuronal cells [e.g., astrocytes (9)]. These truncated Trk-B receptors may serve as high-affinity uptake mechanisms for BDNF and NT-4/5 and restrict actions of BDNF and NT-4/5 to the sites of release (9). The increases in the number of functional Trk-B indicate that upregulation of Trk-B receptor function might be elicited in the cerebral cortex and striatum following cardiac arrest. The increased Trk-B receptors may mediate increased retrograde transport of BDNF and NT-4/5 and contribute to regeneration of neurons after hypoxic-ischemic insults. Increases in the number of truncated Trk-B indicate that there may be an increase in uptake mechanisms and subsequently more efficiency in reuse of BDNF and NT-4/5 released (9). This phenomenon would contribute to the restoration of neurons as well.

In the striatum of rats 3 days after resuscitation, the increased Trk-B receptors may contribute to relative sparing of some neurons in this area of the brain following hypoxic-ischemic insults (2).

In contrast, a downregulation of Trk-B (decreases in immunoreactivities of Trk-B) was observed in the cerebellum of posthypoxic rats. The results suggest that Trk-B may play a lesser role in recovery processes in this particular region of the brain following hypoxic-ischemic insults (10,16).

BDNF is a trophic factor for both cortical neurons (3) and dopaminergic neurons of the substantia nigra (4). It promotes survival and differentiation of dopaminergic neurons, GABAergic neurons of the ventral mesencephalon (5), and cholinergic neurons (8). Furthermore, BDNF activates striatal dopamine and serotonin metabolism (12). It is also known to regulate development of motor neurons (13,18). On the other hand, most of the physiologic roles of NT-4/5 are not known. However, it is known that NT-4/5 activates dopaminergic and GABAergic neurons of the ventral mesencephalon (5) and upregulates the cholinergic phenotypes of developing motor neurons (18). These neuronal systems are known to regulate motor function. In addition, hypoactivity of 5-HT neurotransmission was thought to underlie posthypoxic myoclonus in rats (6,7,17).

Based on these observations, we propose that upregulation of Trk-B receptor function may contribute to the restoration of motor function in posthypoxic rats via several mechanisms. First, it may increase 5-HT neuronal function and promote the survival and regeneration of neurons related to motor function. Second, recovery processes take time (60 days) to complete, and the continued presence of upregulated Trk-B is required [e.g., increases in immunoreactivities of Trk-B were

detected in the cerebral cortex (14 days) and striatum (3 and 14 days) of posthypoxic rats]. Finally, upregulated Trk-B receptor function was also seen in the striatum of rats that recovered (60 days). This phenomenon may be needed for the maintenance of motor function in "recovered" rats.

Our preliminary data indicate that in parallel with Trk-B, increased levels of BDNF and NT-4/5 were detected in the rat brain 14 and 60 days after cardiac arrest and resuscitation. These observations further support our hypotheses.

In short, our current results indicate that regulation of Trk-B may differ in various brain regions and have important roles in recovery processes following hypoxic-ischemic insults to the brain. Both BDNF and NT-4/5 may have therapeutic values in human patients with strokes or posthypoxic myoclonus. Elucidation of mechanisms leading to up- or downregulation of Trk-B receptors in the rat brain following cardiac arrest is currently under way in our laboratory.

ACKNOWLEDGEMENTS

Dr. S. P. Jaw is the recipient of a fellowship award from Myoclonus Research Foundation. The authors are grateful to Van Vuong and Lana Nguyen for their technical assistance.

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