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Astrocyte-Derived Growth Factor (S100β) and Motor Function in Rats Following Cardiac Arrest

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JAW, S. P., D. D. SU AND D. D. TRUONG. *Astrocyte-derived growth factor (S100β) and motor function in rats following cardiac arrest.* PHARMACOL BIOCHEM BEHAV **52**(4) 667-670, 1995.—Following 10-min cardiac arrest and resuscitation, the central serotonergic system and motor function of rats were found to be affected and later on restored. Astrocyte-derived growth factor (S100β) is known to promote survival and neurite outgrowth of serotonergic neurons. In the present study, brain levels of S100β were investigated with quantitative immunoblot analysis at various time points following cardiac arrest. Significant reductions of S100β were found in the cerebral cortex (30%), midbrain (35%), and cerebellum (46%) of rats 3 days postcardiac arrest. In contrast, at 14 and > 45 days, significant increases of S100β were detected in the cerebral cortex (57%; 81%), midbrain (70%; 97%), and cerebellum (84%; 157%). The results indicate that reactive astrocytosis and elevated levels of S100β may participate in the recovery processes following hypoxic-ischemic insults to the brain.

Hypoxia Ischemia S100β Serotonin growth factor Posthypoxic myoclonus

RATS developed posthypoxic stimulus-sensitive myoclonus (involuntary contraction or inhibition of contraction of muscle groups) following 10-min cardiac arrest and resuscitation (12). This phenomenon peaked at 14 days postcardiac arrest. Brain levels of 5-HT and its metabolite, 5-HIAA, and the number of cortical 5-HT_{2A} receptors were found to be reduced in these animals (4,10). In contrast, greater than 45 days, rats no longer had myoclonus upon audiogenic stimulation (12). There was a partial recovery of 5-HT_{2A} receptor density in the cerebral cortex of these rats (4). Immunohistochemical studies further showed that, in comparison with controls, significant reductions of 5-HT immunoreactivities were detected in the frontal-parietal cortex (layer II to V), cerebellum (granular layer), and mesencephalic dorsal raphe nuclei of rats with posthypoxic myoclonus (5). The results indicate that degeneration of 5-HT neurons and loss of their efferent axons and terminals may lead to dysfunction of 5-HT modulatory roles in the motor circuitry, and thus contribute to the disease. Finally, compensatory hyperinnervation of 5-HT fibers were found in rats greater than 45 days following resuscitation (5). These results indicate that hypoactivity of 5-HT neurotransmission, 5-HT_{2A} in particular, may contribute to the motor dysfunction observed initially (4,5). Hyperinnervation of 5-HT fibers in the brain may restore motor function of posthypoxic rats at later time points (5). S100β, a small, dimeric, acidic calcium-binding protein, is known to stimulate neurite outgrowth and promote survival of serotonergic neurons (1,2,7,15). In the present study, we, therefore, investigated roles of S100β in the recovering processes. Brain levels of S100β were examined with quantitative immunoblot analysis. The 5-HT neurons localize in the raphe nuclei of the midbrain and extend their efferent axonal processes to the cerebral cortex and cerebellum, and these brain regions are important in the regulation of motor function; thus, the cerebral cortex, midbrain, and cerebellum were chosed for the present study (5).

METHOD

Animals

Four- to 5-week-old male Sprague-Dawley rats (225-250 g, Zivic Miller, Zelinople, PA) were used. They were maintained for 1 week before surgery on a 12 L : 12 D cycle (lights on

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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).

Cardiac Arrest and Resuscitation Procedures

The procedure for cardiac arrest described by Truong et al. [12] was used. Briefly, prior to 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 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 muscles of the animals and facilitate cardiac arrest. Cardiac arrest 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, the wounds sutured, and the catheters removed.

Behavioral Assessments

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

At various time points (3, 14, and > 45 days) after resuscitation, behavioral scores of rats were assessed before sacrifice. The cerebral cortex, midbrain, and cerebellum were immediately removed, homogenized with 10 volumes of 10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5, and centrifuged at 500 × *g* for 10 min. The supernatant was centrifuged further at 48,000 × g for 10 min, and the subsequent supernatant was collected and stored at −80°C. Protein concentration was measured according to the method of Lowry et al. (8).

Immunoblot Assay

The method described by Van Eldik and Wolchok (13) was modified. The supernatants (40 μg from each sample) were loaded into each well and resolved by 10% SDS-PAGE in 14 × 16 cm slab gels. After resolution in 10% (wt/vol) acrylamide SDS-PAGE, proteins were then transferred onto the nitrocellulose membrane (Schleicher and Schuell, Keene, NH) using Biorad Transblot apparatus (Biorad, Hercules, CA). The membrane was subsequently rinsed briefly with Tris-buffered saline (TBS; 20 mM Tris-HCl, pH 7.5, 500 mM NaCl), incubated in 0.2% (vol/vol) glutaraldehyde in TBS for 45 min at room temperature, and blocked with 5% gelatin in TBS for 2 h at 37°C. The primary antibody (Anti-S100β, 1 : 1,000 dilution, Sigma, St. Louis, MO) in 1% gelatin-TBS was added and incubated for 1 h. 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. The secondary antibody (1 : 1000 dilution, goat antirabbit IgG coupled to horseradish peroxidase, Biorad) 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.

The specificity of S100β protein bands was determined by both comigration of purified S100β (Sigma) with the samples and competition experiments (blocking of immunoreactivities with purified S100β proteins).

The quantitation standard curve was generated from density measurements from 0.01, 0.03, 0.05, 0.07, and 0.1 μg purified S100β proteins.

Image Analysis

The developed immunoblots were scanned with an Microcomputer Image Device (MCID, Imaging Research Inc., Ontario, Canada) densitometer. Background was subtracted by scanning of equivalent sized areas of the nitrocellulose membrane that did not contain immunoreactive proteins.

Statistics

Changes in myoclonus scores and immunoreactivities were analyzed by one-way analyses of variance (ANOVA), followed by Dunnett's *t*-tests. A *p*-value of less than 0.05 was considered significant.

RESULTS

Time Dependency of Presentation of Posthypoxic Myoclonus

The extensive treatments except induction of cardiac arrest did not increase myoclonus scores in rats (data not shown). Futhermore, rats received 1- to 4-min cardiac arrest failed to show any significant increases in myoclonus scores either (data not shown). However, as depicted in Table 1, 3 days post-10-min cardiac arrest and resuscitation, rats exhibited significantly increased myoclonus scores ($n = 7$, $p < 0.01$) compared with those before surgery. This movement disorder continued to be present in rats for over 2 weeks postcardiac arrest (Table 1). The myoclonus scores started to come down from 14 days onward and were back to control levels 45 days postresuscitation (Table 1).

S100β Expression in the Rat Brain

S100β protein levels, as determined by quantitative immunoblot assays, were 0.5 μg/mg protein in the cerebral cortex,

TABLE 1

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

Basal	3 D	14 D	>45 D
52 ± 6	176 ± 5**	188 ± 6**	45 ± 6

The data are expressed as means + SEM. Statistical significance is determined by ANOVA and post hoc Dunnett's *t*-test between basal values assessed from rats before cardiac arrest and those at different days after resuscitation. **$p < 0.01$.

TABLE 2

EFFECTS OF 10-MIN CARDIAC ARREST ON LEVELS OF S100β IN THE RAT BRAIN

	Basal	3 D	14 D	>45 D
Cerebral cortex	0.54 ± 0.02*	0.38 ± 0.01† (70%)‡	0.85 ± 0.04† (157%)	0.98 ± 0.05† (181%)
Midbrain	0.78 ± 0.03	0.51 ± 0.01† (65%)	1.33 ± 0.03† (170%)	1.54 ± 0.14† (197%)
Cerebellum	0.69 ± 0.02	0.37 ± 0.01† (54%)	1.27 ± 0.08† (184%)	1.77 ± 0.16† (257%)

*Values (μg/mg protein) are mean + SEM in triplicate determinations from 6 to 10 rats.
‡% of control values.
†$p < 0.05$, values significantly different from those of the control group as determined by ANOVA followed by Dunnett's t-test.

0.8 μg/mg protein in the midbrain, and 0.7 μg/mg protein in the cerebellum of control rats (Table 2).

The extensive treatments except induction of cardiac arrest did not change S100β protein levels in the rat brain (data not shown). Futhermore, rats received 1- to 4-min cardiac arrest failed to show any significant changes in S100β protein levels in the rat brain either (data not shown).

However, there were significant reductions of S100β in the cerebral cortex (30%), midbrain (35%), and cerebellum (46%) of rats ($n = 7$, $p < 0.05$) 3 days postcardiac arrest. In contrast, at 14 ($n = 9$) and greater than 45 days ($n = 10$), significant elevations of S100β from the control levels were detected in the cerebral cortex (57%; 81%), midbrain (70%; 97%), and cerebellum (84%; 157%) of posthypoxic rats ($p < 0.05$).

DISCUSSION

The initial reduction of S100β may be due to the fact that hypoxic-ischemic insults damage the 5-HT cells (midbrain) and fibers (cerebral cortex and cerebellum) (5), leading to reduction of 5-HT and diminution in astrocytic 5-HT_{1A} receptor activation (10), and subsequently resulting in concommitant decrease in S100β synthesis (3,14).

Increased brain levels of S100β in rats at later time points following cardiac arrest and resuscitation indicate that increased synthesis of S100β and/or reactive astrocytosis in response to neuronal degeneration and death caused by hypoxic-ischemic insults is an event of slow onset and long duration. The elevation of S100β (14 days) took place before 5-HT hyperinnervation in the brain and eventual restoration of motor function (> 45 days) in posthypoxic rats (5,12).

Elevated levels of S100β may enhance 5-HT neurotransmission and promote both astroglial proliferation, and neuronal regeneration and differentiation following hypoxic-ischemic incidents via several mechanisms. First, S100β may promote repair and/or regeneration of 5-HT neurons in the midbrain and neurite outgrowth of 5-HT fiber in the cerebral cortex and cerebellum (5,9,16,17), because increased levels of S100β were found in these areas of the brain. Secondly, S100β is also known to increase 5-HT uptake and astroglia cell population in an autocrine or paracrine fashion (9,11,15). Astrocytosis (increased number of astrocytes) may, in turn, provide more nutrients, growth factors, and signals for surviving neurons to regenerate. Finally, the sprouting of 5-HT efferents and elevated levels of 5-HT (5,10) may provide signals for the regulation of the regeneration, development, differentiation, and neuritic proliferation of surviving non-5-HT neurons (9,16, 17). Hyperinnervation of 5-HT axonal efferents and elevated levels of 5-HT are known to regulate the differentiation and structuring of non-5-HT neurons in the developing cerebral cortex, and these phenomenon disappear in the adult brain (9). However, after hypoxic-ischemic insults to the brain, these events in the developing brain may be reactivated in the recovery processes.

Chronic, but not acute, treatments with 5-HT_{1A} agonists, buspirone and 8-OH-DPAT, attenuated myoclonus in posthypoxic rats (6). These observations further support the hypotheses above. Because hypoactivity of 5-HT neurotransmission may contribute to posthypoxic myoclonus (4,5,10), we propose that induction of S100β may enhance 5-HT neurotransmission, promote astroglial proliferation, and induce both 5-HT and non-5-HT neuronal regeneration and differentiation, thus, contributing to the restoration of motor function in posthypoxic rats. Elucidation of mechanisms for the down-regulation and later induction of S100β both at transcriptional and posttranscriptional levels are currently underway in our laboratory.

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