Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 1, pp. 129–131, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0118-2

Involvement of 5-HT_2 Receptors in Posthypoxic Stimulus-Sensitive Myoclonus in Rats

S. P. JAW,[1] M. J. HUSSONG, R. R. MATSUMOTO AND D. D. TRUONG

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

Received 4 February 1994

JAW, S. P., M. J. HUSSONG, R. R. MATSUMOTO AND D. D. TRUONG. *Involvement of 5-HT_2 receptors in posthypoxic stimulus-sensitive myoclonus in rats.* PHARMACOL BIOCHEM BEHAV **49**(1) 129–131, 1994.—We have previously reported that rats exhibited audiogenic myoclonus at 3 days after cardiac arrest. This phenomenon peaked at 14 days, gradually tapered off at older ages, and disappeared in most rats by 60 days following cardiac arrest. Because treatment with the 5-HT_2-selective agonist, (±)-1-2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI) significantly attenuated audiogenic myoclonus in these postcardiac-arrest rats, the involvement of 5-HT_2 receptors in posthypoxic stimulus-sensitive myoclonus was suggested. In the current study, we, therefore, examined the binding properties of 5-HT_2 receptors in the rat bain at various time points following cardiac arrest. The affinity constant of [^{3}H]ketanserin binding to 5-HT_2 receptors in cortical membranes of rats did not change. In contrast, B_{max} values were found to be reduced at 3 and 14 days after cardiac arrest with some recovery after 60 days. Taken together with previous results, these results indicate that hypoactivity of central 5-HT_2 neurotransmission may underlie the development of posthypoxic stimulus-sensitive myoclonus in rats.

Serotonin Ketanserin DOI 5-HT_2 Receptors Cardiac arrest Hypoxia Ischemia Myoclonus

MYOCLONUS depicts series of a sudden, synchronous, involuntary contraction of muscle groups (3). Posthypoxic myoclonus (Lance-Adams syndrome) was first described by Lance and Adams (6). Brain levels of serotonin (5-hydroxytryptamine, 5-HT), and its metabolite, 5-hydroxyindoleaceticacid (5-HIAA), were found to be reduced in a variety of myoclonic states, including posthypoxic and posttraumatic intention myoclonus, progressive myoclonic epilepsy, and essential myoclonus (15). In these cases, patients improved when treated with 5-hydroxytryptophan (5-HTP, 5-HT precursor) and drugs that enhance 5-HT activity (1,2).

A cardiac arrest rat model of posthypoxic myoclonus was recently described by Truong et al. (14). 5-HT and 5-HIAA in the brain were also found to be reduced in postcardiac-arrest-myoclonic rats (8). Symptoms of audiogenic myoclonus were relieved in these animals when treated with 5-HTP, valproic acid, and clonazepam (14); these drugs are known to enhance serotonergic neurotransmission. These observations in human patients as well as in the animal model indicate that hypoactivity of central serotonergic neurotransmisson may contribute to posthypoxic myoclonus.

The questions then arises as to which 5-HT receptor subtype(s) mediate the observed effects and what changes occur at 5-HT receptors both physically and functionally in posthypoxic-myoclonic rats. Because treatment with DOI, a 5-HT_2 receptor agonist, attenuated audiogenic myoclonus in postcardiac-arrest rats, in the present study, we examined the binding properties of 5-HT_2 receptors in cortical membranes from postcardiac-arrest rats. The cortex was chosen for these studies because it receives afferent projections from the raphe nucleus (5-HT neurons) in the midbrain, is involved in the motor circuitry of the brain, and is thought to be influential in the pathophysiology of many types of myoclonus (11).

METHOD

Animals

Four- to five-week-old male Sprague–Dawley rats (125–150 g, Zivic Miller, Zelinople, PA) were used. 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

The procedure for cardiac arrest described by Truong et al. (14) 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 μg/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.

Membrane Preparation

As reported previously (14), some rats developed seizures, spontaneous myoclonus, and audiogenic myoclonus in the first 2 days after cardiac arrest. The first two phenomena disappeared in 3-day postcardiac-arrest rats, at the same time, audiogenic myoclonus become prominent in all rats. Posthypoxic stimulus-sensitive myoclonus persisted and peaked at 14 days following cardiac arrest. However, at longer time points after the arrest, rats became less sensitive to audiogenic stimulation. By 45 days following cardiac arrest, rats failed to show myoclonus upon stimulation. In the current study, we, therefore, selected rats at 3, 14, and an average of 60 days after cardiac arrest and determined binding properties of cerebral cortical 5-HT_2 receptors in these rats.

Rats were decapitated 3 days, 14 days, and average 60 days after cardiac arrest. Cortices from five to eight rats were dissected out on ice, pooled, homogenized in ice-cold 50 mM Tris-HCl, 0.5 mM EDTA Na_2, 5 mM $MgCl_2$, pH 7.4 (1 : 10 weight/volume), and centrifuged at 30,000 × g for 15 min at 4°C. The pellet was resuspended in ice-cold deionized H_2O and kept on ice for 20 min. The suspension was then centrifuged at 30,000 × g for 15 min at 4°C and washed three times (30,000 × g, 15 min). Between the second and third wash, the suspension was incubated at 26°C for 60 min to degrade endogenous ligands.

Receptor Binding Assay

Saturation binding assays for 5-HT_2 receptors were carried out in triplicate with [^{3}H]ketanserin (New England Nuclear, Boston, MA, 63.7 Ci/mmol). Various concentrations of [^{3}H]ketanserin (0.05–2 nM) were incubated for 60 min at 26°C with a final volume of 0.5 ml of membrane preparation in 50 mM Tris-HCl, 0.5 mM EDTA Na_2, 10 mM $MgCl_2$, 0.1% ascorbate, 10 μM pargyline, pH 7.4. Nonspecific binding was determined in the presence of 1 μM ketanserin (Sigma, St. Louis, MO). Incubations were terminated by collecting the membranes on glass fiber filters (Schleicher and Schuell, Keene, NH) using a Brandel cell harvester. Filters were then washed three times with 5 ml of 50 mM Tris-HCl, pH 7.4, at 4°C and transferred to scintillation vials. Ultima Gold (5 ml) (Packard Instrument Company, Meriden, CT) was added to the vials and the radioactivity was determined by liquid scintillation spectrometry. The amount of membrane protein used in each assay was in the range of 0.5–1.0 mg/ml, as determined by the method of Lowry et al. (7).

Statistics

Changes in myoclonus scores and receptor binding parameters 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. Saturation experiments were analyzed by the EBDA program of McPherson (9). Nonlinear regression calculated from Scatchard analysis was further analyzed by the Ligand program of Munson (10).

RESULTS

Time Dependency of the Expression of Posthypoxic Stimulus-Sensitive Myoclonus

Figure 1 depicts the myoclonus scores measured from rats at different time points since the cardiac arrest. The myoclonus scores for rats at 3 (n = 5) or 14 days (n = 6) after cardiac arrest were significantly higher, $F(3, 25) = 15.94$, $p < 0.01$, than those of the control group (n = 8). However, scores for rats at longer times after cardiac arrest (average 60 days) (n = 7) were not significantly different from those of the control rats.

Radioligand Binding Studies

At 3 and 14 days after cardiac arrest, the number of 5-HT_2 receptors as labelled by [^{3}H]ketanserin was decreased to almost one-half of those in controls, $F(3, 11) = 16.79$, $p < 0.01$, (Table 1). By 60 days after cardiac arrest, the number of 5-HT_2 receptors increased 45% from those of 14-day post-

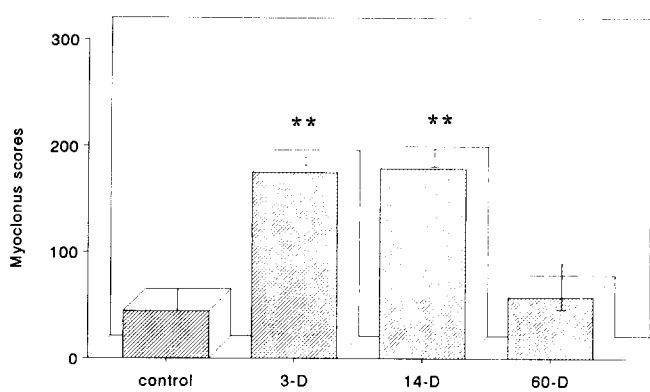

FIG. 1. Audiogenic myoclonus scores of normal rats and animals 3, 14, and 60 days after cardiac arrest. **Significant at $p < 0.01$, when values compared with those of the control group as determined by Dunnett's *t*-test.

TABLE 1

5-HT$_2$ RECEPTOR BINDING PARAMETERS IN CORTICAL MEMBRANES OF RATS

	K_D (nM)	B_{max} (fmol/mg protein)
I. Control	[a]1.63 ± 0.42	[a]246.5 ± 18.7 (100 %)[c]
II. 3 days[b]	1.31 ± 0.16	127.6 ± 8.8* (51.8 %)
III. 14 days	1.74 ± 0.29	117.4 ± 12.1* (47.6 %)
VI. 60 days	1.56 ± 0.05	168.9 ± 14.4* (68.4 %)

[a]Values are mean ± SEM in triplicate determinations from five to eight rats.

[b]Numbers of days after cardiac arrest.

[c]Percent values of those of the control groups.

*Significant at $p < 0.01$, when values compared with those of the control group as determined by Dunnett's t-test.

cardiac-arrest rats ($p < 0.01$). However, it was still below the precardiac-arrest level (Table 1). In contrast, no significant changes were found in the affinity constant of 5-HT$_2$ receptors among these rats (Table 1).

DISCUSSION

In the current study, 5-HT$_2$ receptors in the rat cortex were reduced to one-half of control levels at 3 and 14 days after cardiac arrest. During this time, animals developed full-blown stimulus-sensitive myoclonus. In contrast, by 60 days after cardiac arrest, the number of 5-HT$_2$ receptors in the rat cortex increased from the low level of rats at 14 days since cardiac arrest. At the same time, older rats no longer showed myoclonus upon audiogenic stimulation.

Several factors may have contributed to the loss/reduction of 5-HT$_2$ receptors in rats 3 and 14 days after cardiac arrest. During a cardiac arrest, brain levels of 5-HT are known to be increased (13), and acute increased concentrations of an agonist would rapidly sequester and internalize 5-HT$_2$ receptors (12). However, these mechanisms alone would not acount for the long-term changes observed in the present study. Because 5-HT$_2$ heteroreceptors appear on some γ-amino-butyric-acid (GABA)-containing neurons, and loss of GABA neurons were seen in rats following cardiac arrest (5), loss of GABA neurons together with membrane-bound 5-HT$_2$ heteroreceptors may account for the reduced number of 5-HT$_2$ receptors observed in the present study. Furthermore, because GABA afferents to the raphe nucleus (5-HT neurons) exist (4), loss of these GABA inhibitory inputs due to cardiac arrest would be expected to increase the firing of raphe neurons, subsequently increasing impulse flow-dependent 5-HT release. The increased steady level of 5-HT would then be expected to reduce the number of postsynaptic 5-HT$_2$ receptors as observed in the present study.

In rats with longer recovery times after cardiac arrest (average 60 days), the number of 5-HT$_2$ receptors was found increased from the low level of rats at 14 days since cardiac arrest. This may be due to compensatory increases in de novo synthesis of receptors by existing neurons, regrowth of new neurons, or repairs and restoration of damaged neurons. Further experiments are required to explore mechanisms for the observed alterations of 5-HT$_2$ receptor population during various time points after cardiac arrest.

In conclusion, our present results support the hypothesis that hypoactivity of 5-HT$_2$ neurotransmission may contribute to posthypoxic myoclonus. The roles of other 5-HT receptor subtypes are currently under investigation in our laboratory.

ACKNOWLEDGEMENTS

This work was supported by a grant from Myoclonus Research Foundation. We are grateful to Avneet Singh and Lana Nguyen for their technical assistance.

REFERENCES

1. Deahl, M.; Trimble, M. Serotonin reuptake inhibitors, epilepsy and myoclonus. Br. J. Psychol. 159:433–435; 1991.
2. De Lean, J.; Richardson, J. C.; Hornykiewicz, O. Beneficial effects of serotonin precursors in postanoxic action myoclonus. Neurology 26:863–868; 1976.
3. Fahn, S. Posthypoxic action myoclonus: Literature review and update. Adv. Neurol. 43:157–169; 1986.
4. Harandi, M.; Aguera, M.; Gamrani, H.; Didier, M.; Maitre, M.; Calas, A.; Belin, M.F. γ-Aminobutyric acid and 5-hydroxytryptamine interrelationship in the rat nucleus raphe dorsalis: Combination of radioautographic and immunocytochemical techniques at light and electron microscopy levels. Neuroscience 21:237–251; 1987.
5. Kawai, K.; Nitecka, L.; Ruetzler, C.A.; Nagashima, B.; Joo, F.; Mies, G.; Nowak, T. S.; Saito, N.; Lohr, J. M.; Klatzo, I. Global cerebral ischemia associated with cardiac arrest in the rat: I. Dynamics of early neuronal changes. J. Cereb. Blood Flow Metab. 12:238–249; 1992.
6. Lance, J. W.; Adams, R. D. The syndrome of intention or action myoclonus as a sequel to hypoxic encephalopathy. Brain 86:111–136; 1963.
7. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
8. Matsumoto, R. R.; Aziz, N.; Truong, D. D. Association between brain indoleamine levels and severity of a cardiac arrest induced movement disorder. (submitted).
9. Mcpherson, G. A. A practical approach to the analysis of radioligand binding experiments. Comput. Programs Biomed. 17:107–113; 1983.
10. Munson, P. J.; Rodbard, D. Ligand: A versatile computerized approach for characterization of ligand binding systems. Anal. Biochem. 107:220–239; 1980.
11. Obeso, J. A.; Marsden, C. D. Different clinical presentations of myoclonus. Parkinson's diseases and movement disorders. Baltimore: Urban and Schwarzenberg; 1988:263–274.
12. Rahman, S.; Neuman, R. S. Multiple mechanisms of serotonin 5-HT$_2$ receptor desensitization. Eur. J. Pharmacol. 238:173–180; 1993.
13. Sarna, G. S.; Obrenovitch, T. P.; Matsumoto, T.; Symon, L.; Curzon, G. Effect of transient cerebral ischaemia and cardiac arrest on brain extracellular dopamine and serotonin as determined by in vivo dialysis in the rat. J. Neurochem. 55:937–940; 1990.
14. Truong, D. D.; Matsumoto, R. R.; Schwartz, P. H.; Hussong, M. J.; Wasterlain, C. G. A novel rat cardiac arrest model of posthypoxic myoclonus, Movement Disord. 9:201–206; 1994.
15. Van Woert, M. H.; Hwang, E. C. Role of brain serotonin in myoclonus. Neurotransmitt. Seizures Epilepsy 26:239–246; 1981.