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Association Between Brain Indole Levels and Severity of Posthypoxic Myoclonus in Rats

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MATSUMOTO, R. R., N. AZIZ AND D. D. TRUONG. *Association between brain indole levels and severity of post-hypoxic myoclonus in rats.* PHARMACOL BIOCHEM BEHAV **50**(4) 533-538, 1995.—We have previously reported the presence of posthypoxic, audiogenic myoclonus in rats after cardiac arrest and the ability of the 5-HT precursor, 5-HTP, to attenuate these muscle jerks. In addition, we have recently shown that 5-HT_2 and 5-HT_3 agonists can reduce the severity of myoclonus in these animals, suggesting a deficiency in serotonergic neurotransmission. In the present study, the levels of 5-HTP, 5-HT, and 5-HIAA were measured in seven regions of the brain in myoclonic and normal rats to identify the areas of the brain in which a serotonergic dysfunction resides. Similar to previous studies, we observed pronounced posthypoxic, audiogenic myoclonus 3 and 14 days after resuscitation from cardiac arrest, with a resolution of the abnormal movements by 45 days postarrest. HPLC measurements revealed significant changes in indole levels in the following areas of the brain: cortical 5-HIAA, striatal 5-HT, striatal 5-HIAA, hippocampal 5-HT, mesencephalic 5-HIAA, myelencephalic 5-HT, myelencephalic 5-HIAA, cerebellar 5-HTP, and cerebellar 5-HT. The changes in striatal 5-HT, cortical 5-HIAA, and mesencephalic 5-HIAA appear most relevant to the pathophysiology of posthypoxic myoclonus because regression analyses showed significant correlations between the myoclonus scores of the animals and the levels of these indoles. Based on the observed pattern of results, we postulate a dysfunction in serotonergic lateral (cortical) and far lateral (extrapyramidal) ascending pathways in posthypoxic myoclonus.

Myoclonus Serotonin Hypoxia Ischemia HPLC

MYOCLONUS is defined as sudden, brief, shock-like involuntary movements caused by active muscular contractions or inhibitions (8). It can be precipitated by a variety of pathologic conditions affecting the central nervous system, including cardiac arrest. Myoclonus resulting from cardiac arrest is considered a type of posthypoxic myoclonus (9) and was first described in humans by Lance and Adams (23). Although ischemia and other factors contribute to myoclonus after cardiac arrest, for the sake of consistency with the clinical literature we will continue to refer to the abnormality in our rats as posthypoxic myoclonus.

Although little is known about the neuropathology associated with posthypoxic myoclonus, previous studies have suggested a role for serotonin in the disorder (24,29,37). The serotonin precursors 5-HTP and L-tryptophan are among the most effective treatments for posthypoxic myoclonus in humans (7,9,11), and depressed levels of 5-HIAA, a major metabolite of serotonin, are found in the CSF of some patients with posthypoxic myoclonus (3,12,35,38). Despite evidence for a serotonin dysfunction in posthypoxic myoclonus, further studies were difficult in humans, and until recently, no animal model was available to study the disorder.

We recently described a cardiac arrest-induced animal model of posthypoxic myoclonus (36). The etiology, time course, and pharmacology associated with the involuntary muscle jerks in these cardiac-arrested rats were consistent with myoclonus and appeared distinguishable from seizures and startle responses (36). The myoclonus in these animals was attenuated with typical antimyoclonic drugs such as 5-HTP, valproate, and clonazepam (36); all of these drugs posses the ability to affect serotonergic neurotransmission (1,4,25, 30,39). As a result of testing with selective serotonergic ligands, we have recently found that 5-HT_2 and 5-HT_3 agonists can also attenuate posthypoxic myoclonus in our rats. Because observations in our animal model as well as in humans support the existence of a serotonergic dysfunction in posthypoxic myoclonus, in the present study, we measured the levels of 5-HTP, 5-HT, and 5-HIAA in seven regions of the brain in

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rats with and without posthypoxic myoclonus to identify the specific brain areas and systems that are affected in the disorder.

METHOD

Cardiac Arrest Surgeries

The procedures for the cardiac arrest surgeries were as previously described (36) and represent an adaptation of models originally developed by others (2,5). All procedures were approved by the University of California Irvine Animal Care and Use Committee. Briefly, male Sprague-Dawley rats (Zivic Miller, Zelinople, PA; 200-250 g) were fasted for 12-24 h prior to surgery. The animals were anesthetized with 100 mg/kg ketamine and 0.4 mg/kg atropine. The rats were tracheotomized and attached to a ventilator (ventilator settings: 425 cc/min NO_2, 175 cc/min O_2, 60 strokes/min, 10 ml/kg, 5 cm H_2O PEEP). A femoral artery and vein were catherized for the measurement of blood pressure and administration of drugs, respectively. Electrocardiogram and blood pressure were monitored continuously throughout the rest of the procedure. Rats were paralyzed with succinylcholine to facilitate the arrest. Cardiac arrest was performed by transthoracic intracardial injection of 0.4 ml ice-cold KCl (1%) and cessation of ventilation. Resuscitation began 8 min after the arrest by resuming ventilation (ventilator settings: 100% O_2, 100 strokes/min), manual thoracic compressions and IV injection of 10 μg/kg epinephrine and 4 mEq/kg sodium bicarbonate. Rats were weaned from the ventilator over 2-4 h, the wounds sutured, and the catheters removed.

Behavioral Testing

Although the cardiac-arrested rats exhibited spontaneous myoclonus, audiogenic myoclonus was used as the behavioral endpoint because it persists for a longer period of time after resuscitation from cardiac arrest (36), and the time-locked nature of the response to auditory stimuli facilitates the quantification of the severity of the abnormal movements. On the day the animals were sacrificed, audiogenic myoclonus was measured in the rats using a method described previously (36). Briefly, rats were placed in a plastic test cage (42 × 21 × 21 cm) and presented with a series of 45 clicks generated by a metronome (1 Hz, 95 dB, 40 ms when measured at the level of the rat). The response of the rats to each click was scored by a trained observer as follows: 0 = no jerk; 1 = ear twitch only; 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 sum of the scores over the 45 clicks yielded the total myoclonus score for that rat. Thus, the maximum score was 225 and the minimum was 0; in practice, the minimum score was rarely below 45. Although some of the rats in the 3-day postop group were in a weakened state at the time of testing, all of the animals had recovered sufficiently from surgery that they were able to move freely, in such a way that the scoring was not compromised.

In our initial report, the average score for a normal, noncardiac-arrested rat was 73 ± 16, while the average score for a cardiac-arrested rat was significantly higher at 157 ± 13 (36). The scoring system was implemented to avoid a floor effect in earlier pharmacological studies (it was possible that a drug might incapacitate a cardiac-arrested rat to the point where it was incapable of responding, thus yielding a score that was significantly lower than a normal rat). Although pharmacological testing was not performed in the studies herein, the scoring system was retained to facilitate comparisons to results obtained in other experiments in which this model was used.

Preparation of Brain Tissue

Normal and cardiac-arrested rats (3, 14, and > 45 days postarrest) were decapitated and the following brain regions dissected and immediately frozen on dry ice: cerebellum, striatum, hippocampus, cortex, diencephalon (minus striatum and hippocampus), mesencephalon (regions inclusive of and underlying colliculi), and myelencephalon. The specified time periods for sacrifice of the cardiac-arrested rats were chosen based on previous studies (36) and represent times when a) both spontaneous and audiogenic myoclonus are present (3 days), b) the audiogenic myoclonus is at or near its peak (14 days), and c) the myoclonus scores of the animals are subnormal (> 45 days).

Tissue samples were prepared from individual animals (controls n = 9, 3 days n = 5, 14 days n = 7, > 45 days n = 7). For the extractions, regional brain tissues were thoroughly disrupted on ice in 9 vol of 0.1 M perchloric acid (containing 0.1 mM EDTA and 0.4 mM Na_2SO_3), and 100 μl of 10 nmol/ml isoproterenol (internal standard) using a tissue tearor (Biospec Products, Bartlesville, OK). The homogenates were centrifuged at 14,000 rpm at 4°C for 15 min in a high-speed microcentrifuge. The supernatant, thus obtained, was filtered through a 0.2 μm nylon membrane in a microfiltration tube using the microcentrifuge at 5000 rpm, at 4°C for 5 min. The filtrate (100 μl) was injected on the HPLC analytical column.

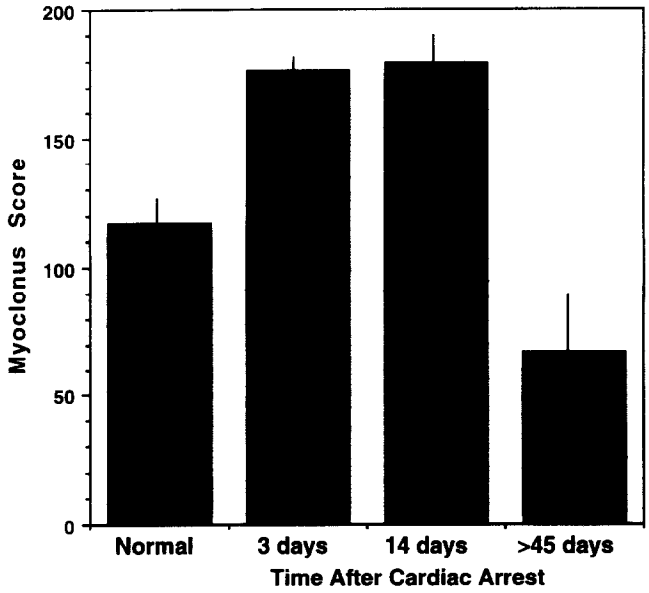

FIG. 1. Behavioral scores of rats prior to sacrifice for the HPLC portion of the study. There were significant differences between the myoclonus scores (mean ± SEM) of normal rats and cardiac-arrested animals 3, 14, and > 45 days postarrest ($p < 0.001$). The pronounced audiogenic, posthypoxic myoclonus exhibited by rats 3 and 14 days after cardiac arrest was reflected in the higher myoclonus scores of these rats as compared to normal animals (Dunnett tests $p < 0.05$ for 3 days, $p < 0.01$ for 14 days postarrest). By 45 days postarrest, these abnormal movements were no longer exhibited by cardiac-arrested rats, with the animals showing somewhat of a lesser response to audiogenic stimulation than normal animals.

Measurement of Indoles

The levels of 5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT), and 5-hydroxyindole acetic acid (5-HIAA) were measured in different brain regions of normal and cardiac-arrested rats using HPLC with electrochemical detection. An isocratic system was used for the separation of compounds; isoproterenol was used as the internal standard. The mobile phase consisted of 100 mM sodium phosphate, 0.1 mM EDTA, 1.0 mM heptanesulfonic acid, 5% v/v acetonitrile, and 0.01% triethylamine (pH 3.0, adjusted with phosphoric acid). The solution was filtered and degassed for at least 30 min before it was passed through the column (flow rate = 1.2 ml/min).

Our HPLC system consisted of a Rainin solvent delivery pump (Model HPXL), a six-port injector (Model 7125, Rheodyne, Berkeley, CA), an analytical column (Ultrasphere ODS-C18, 7.5 cm × 4.6 mm i.d., particle size 3 μm, Beckman, USA), and an electrochemical detector (Coulochem ESA detector Model 5100A, Severn Analytical Ltd., UK), with the electrode (Model 5011) set at +0.45 V, with respect to a paladium reference electrode. The guard cell (Model 5020) was set at 0.55 V. The signals were processed on a Mcintosh Plus computer using the Dynamax HPLC Method Manager (Rainin, Worburn, MA).

Peaks were identified by comparing the retention time of each peak from the sample solution to that of each peak in the standard solution, and by superimposing the chromatograms of the samples spiked with known amounts of the standards. The levels of indoles in each brain sample were calculated by our software from comparisons of sample peak area with internal standard peak area.

A linear relation ($r > 0.99$) was observed for all substances measured in this study within a concentration range of 10 nmol/ml to 10 pmol/ml. In addition, the recovery of different monoamines, metabolites, and precursors through the extraction and filtration process was 94% or better.

Statistics

One-way analyses of variance (ANOVA), followed by post hoc Dunnett tests, were used to determine whether there were significant differences between the levels of indoles in various brain regions of normal rats and cardiac-arrested animals 3, 14, and > 45 days postarrest. In addition, changes in turnover were assessed by comparing the ratios of 5-HIAA : 5-HT.

TABLE 1

LEVELS OF INDOLEAMINES IN BRAIN REGIONS OF NORMAL AND CARDIAC ARRESTED RATS

	Control	3 Days	14 Days	> 45 Days
Cortex				
5-HTP	57 ± 12	39 ± 11	31 ± 8	38 ± 9
5-HT	152 ± 23	226 ± 62	231 ± 27	340 ± 79
5-HIAA	500 ± 34	764 ± 92	476 ± 61	978 ± 115*
Diencephalon				
5-HTP	75 ± 8	94 ± 17	58 ± 15	104 ± 21
5-HT	317 ± 37	431 ± 128	437 ± 92	563 ± 141
5-HIAA	1062 ± 62	1195 ± 109	978 ± 51	1131 ± 64
Striatum				
5-HTP	66 ± 11	63 ± 12	104 ± 29	100 ± 15
5-HT	235 ± 15	188 ± 11	246 ± 37	392 ± 27*
5-HIAA	561 ± 90	424 ± 36	237 ± 48*	518 ± 84
Hippocampus				
5-HTP	15 ± 1	10 ± 2	9 ± 1	10 ± 2
5-HT	150 ± 16	199 ± 13	125 ± 18	206 ± 24
5-HIAA	1038 ± 83	1100 ± 63	890 ± 46	993 ± 99
Mesencephalon				
5-HTP	22 ± 6	26 ± 15	29 ± 13	69 ± 24
5-HT	454 ± 38	391 ± 71	403 ± 116	606 ± 55
5-HIAA	1561 ± 143	2952 ± 212*	2211 ± 172*	1895 ± 138
Myelencephalon				
5-HTP	69 ± 12	75 ± 23	53 ± 14	100 ± 20
5-HT	609 ± 41	578 ± 36	462 ± 27	708 ± 58
5-HIAA	711 ± 42	986 ± 115*	602 ± 63	925 ± 75
Cerebellum				
5-HTP	28 ± 4	16 ± 2*	17 ± 2*	16 ± 1
5-HT	85 ± 7	55 ± 5*	80 ± 7	62 ± 7
5-HIAA	167 ± 15	177 ± 9	179 ± 25	158 ± 19

The levels of indoles (5-HTP, 5-HT, and 5-HIAA) were determined by HPLC with electrochemical detection in seven brain regions of normal animals (control) and in cardiac arrested rats (3, 14, and > 45 days postarrest). One-way analyses of variance were used to determine whether indole levels in a particular brain region differed significantly between the groups. Post hoc Dunnett tests were then used to determine which cardiac arrested groups differed significantly from normal controls. Significant effects with the post hoc tests are indicated in the table with an asterisk ($p < 0.05$). Values in the table represent the mean ± SEM of indole levels (in ng/g wet tissue weight).

In those regions of the brain where there was a significant change in the levels of 5-HTP, 5-HT, or 5-HIAA in cardiac-arrested rats, regression analysis was used to determine whether a relationship existed between the myoclonus scores of the animals and the levels of the affected indoles. Although the myoclonus scores represent the sum of ordinal variables, the wide range of possible scores (0–225), together with the interval nature of the HPLC data, justified the use of parametric statistics for these comparisons. For all of the statistical tests, a significance level of $p < 0.05$ was used, and evaluations were made according to the methods described by Hays (16) and Keppel (21).

RESULTS

Behavior

The myoclonus scores of the animals differed significantly between the groups, $F(3, 31) = 10.14, p < 0.001$; Fig. 1. At 3 and 14 days after resuscitation, post hoc Dunnett tests revealed that the animals had significantly higher myoclonus scores than normal rats ($p < 0.05$ for 3 days; $p < 0.01$ for 14 days), reflecting the fact that at these time points, the cardiac-arrested rats exhibited pronounced audiogenic myoclonus. Consistent with previously reported results, by 45 days after resuscitation the cardiac-arrested rats no longer displayed posthypoxic, audiogenic myoclonus (36).

Indole Levels

The levels of indoles in various brain regions of normal and cardiac-arrested rats are summarized in Table 1. There were significant changes in the levels of the following indoles in the indicated brain regions after cardiac arrest: cortical 5-HIAA, $F(3, 24) = 9.63, p < 0.001$, striatal 5-HT, $F(3, 24) = 7.52, p < 0.001$, striatal 5-HIAA, $F(3, 24) = 3.72, p < 0.03$, hippocampal 5-HT, $F(3, 24) = 4.16, p < 0.02$, mesencephalic 5-HIAA, $F(3, 24) = 18.60, p < 0.001$, myelencephalic 5-HT, $F(3, 24) = 5.48, p < 0.005$, myelencephalic 5-HIAA, $F(3, 24) = 6.30, p < 0.003$, cerebellar 5-HTP, $F(3, 24) = 4.01, p < 0.02$, and cerebellar 5-HT, $F(3, 24) = 4.49, p < 0.02$. Post hoc comparisons for these changes are summarized in Table 1. In addition, significant changes in turnover, as measured by the ratio of 5-HIAA : 5-HT, were found in the following regions: striatum, $F(3, 24) = 3.54, p < 0.03$, mesencephalon, $F(3, 24) = 8.06, p < 0.001$, and cerebellum, $F(3, 24) = 6.53, p < 0.003$. All other comparisons were not statistically significant (ANOVA). In those areas of the brain exhibiting significant differences in the levels of indoles after cardiac arrest, the following changes were shown to be correlated with the myoclonus scores of the cardiac-arrested animals (Fig. 2): cortical 5-HIAA ($r = 0.49, t = -2.26, p < 0.04$), striatal 5-HT ($r = 0.70, t = -3.92, p < 0.001$), and mesencephalic 5-HIAA ($r = 0.51, t = 2.37, p < 0.04$).

DISCUSSION

The findings herein support previous suggestions that a serotonergic dysfunction is associated with posthypoxic myoclonus. Significant inverse correlations were found between the severity of audiogenic myoclonus in cardiac-arrested rats and the levels of striatal 5-HT and cortical 5-HIAA (i.e., abnormally low levels of these indoles were associated with increased severity of posthypoxic myoclonus). Although correlation does not equal causation, the possibility that such a deficiency in serotonergic tone would contribute to posthypoxic myoclonus is supported by the ability of 5-HT_2 and 5-HT_3

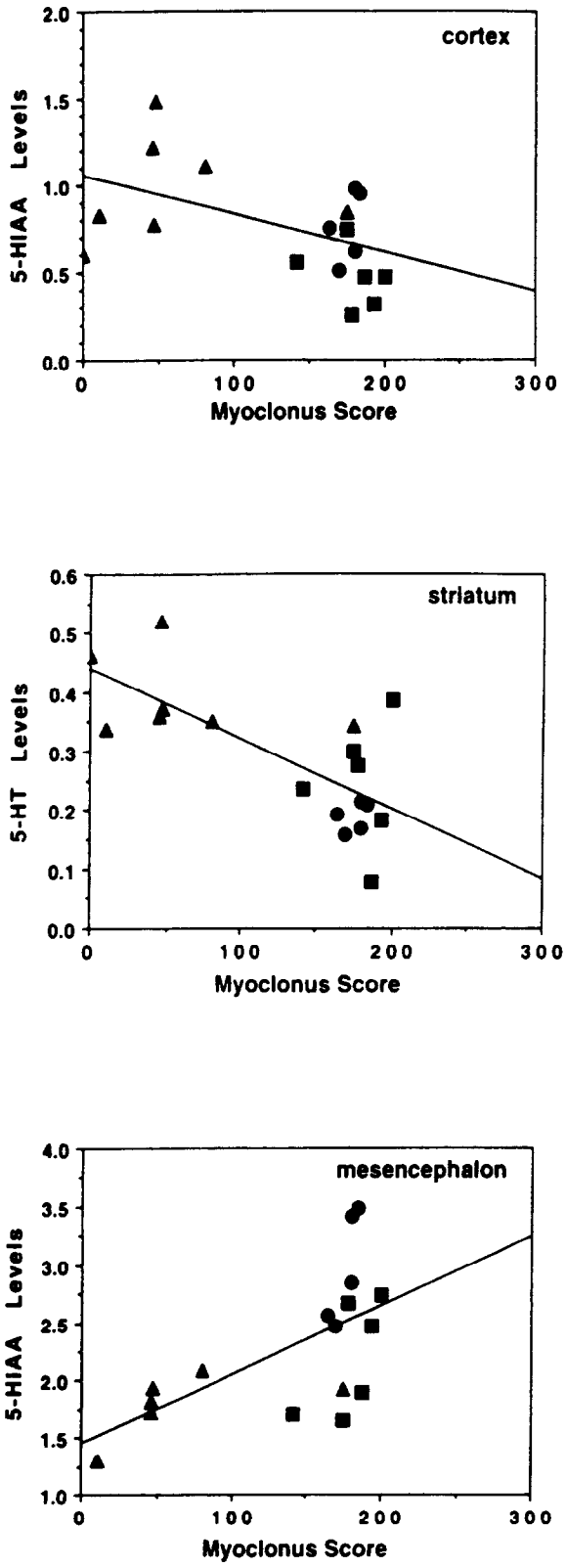

FIG. 2. Significant correlations ($p < 0.05$) between brain indole levels and the myoclonus scores of cardiac-arrested rats (● = 3 days, ■ = 14 days, ▲ = > 45 days postarrest). In each panel, the myoclonus scores of individual animals are paired with their respective brain indole levels. Indole levels are in μg/g wet tissue weight.

agonists (DOI and M-chloro-phenyl-biguanide) and a 5-HT precursor (5-HTP) to attenuate the audiogenic myoclonus exhibited by these animals (18,36).

These changes are not thought to represent nonspecific effects associated with the cardiac arrest because the changes were localized to regions of the brain that have previously been implicated in other types of myoclonus. The cortex is involved in many types of myoclonus, including cortical reflex myoclonus (13,32), and perturbation of the striatum is capable of evoking myoclonus in experimental animals (27,33). Furthermore, autoradiographic studies have shown that 5-HT_2 and 5-HT_3 receptors are found in the cortex and striatum (17,22,28) and we have recently demonstrated abnormal levels of 5-HT_2 receptors in the cortex of cardiac-arrested rats with posthypoxic myoclonus (20). Taken together, the data suggest that abnormalities in the cortex and striatum may, in fact, contribute to the pathophysiology of posthypoxic myoclonus.

In contrast to the pattern shown in the rest of the brain where there was a tendency for decreases in indole levels to appear after cardiac arrest, in the mesencephalon, high levels of 5-HIAA were significantly associated with severe myoclonus. We postulate that the increased 5-HIAA levels in the mesencephalon reflect enhanced catabolism of intraneuronal serotonin (40), because there was no concomitant evidence of increased synthesis or release of serotonin elsewhere in the brain. Such an explanation is consistent with the reduction in serotonergic tone in a patient with posthypoxic myoclonus whose postmortem brain exhibited no loss of serotonergic neurons in the raphe (6). Although modern means of detecting raphe neurons were not available when that particular postmortem brain was evaluated, the observation is likely to be valid because no loss of raphe neurons has been observed in the postmortem brain of many subsequent patients exhibiting posthypoxic myoclonus (15) nor in the cardiac-arrested rats (31). It is, therefore, possible that increased catabolism of intraneuronal serotonin in the dorsal and medial raphe contributes to the reduction in serotonergic tone that is associated with posthypoxic myoclonus. Furthermore, because the lateral and far lateral serotonergic pathways terminate in the cortex and striatum, respectively (10), it is possible that they play a relevant role in myoclonus.

As with any study of this type in which the levels of indoles are measured postmortem, the results must be interpreted with caution. Caveats that must be considered include the fact that decapitation, the method of sacrifice used in this study, has been shown to alter indole levels (19). Subsequent to death, changes in enzymatic activity can also alter indole levels. However, it is unlikely that these two factors significantly influence the interpretation of our data because control animals also underwent similar procedures as experimental rats. A consideration of more relevance, however, is the observation that serotonin is normally synthesized in excess of functional need. Because serotonin may be degraded by monoamine oxidase prior to release (40), the measured levels of both 5-HT and 5-HIAA in the various brain regions do not necessarily reflect transsynaptic functional activity. Even considering this possibility, our data, taken together with the existing literature, is consistent with a serotonergic hypoactivity in posthypoxic myoclonus.

Other changes in indole levels that do not appear related to myoclonus were also observed in our cardiac-arrested animals. These latter changes may have resulted from events associated with the cardiac arrest itself (e.g., ischemia, hypoxia), neurological sequelae following the arrest (e.g., seizures, hindlimb hypertonus), or restitution of function after the arrest (e.g., neuronal sprouting). Because depletions of indole levels during the early phases after an ischemic event have been reported by a number of laboratories (14,26,34), it is possible that observed decreases in our data are also caused, at least in part, by ischemic events associated with the cardiac arrest. Further studies to define the contribution of this and other factors to the nonmyoclonus-related changes in indole levels are currently underway in our laboratory.

In conclusion, our present results are consistent with the hypothesis that hypoactivity of serotonergic neurotransmission contributes to posthypoxic myoclonus. In particular, dysfunctions in the lateral (cortical) and far lateral (extrapyramidal) serotonergic pathways appear relevant to the disorder.

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REFERENCES

1. Biggs, C. S.; Pearce, B. R.; Fowler, L. J.; Whitton, P. S. Regional effects of sodium valproate on extracellular concentrations of 5-hydroxytryptamine, dopamine, and their metabolites in the rat brain: An in vivo microdialysis study. J. Neurochem. 59:1702–1708; 1992.
2. Blomqvist, P.; Wieloch, T. Ischemic brain damage in rats following cardiac arrest using a long-term recovery model. J. Cereb. Blood Flow Metab. 5:420–431; 1985.
3. Chadwick, D.; Hallett, M.; Harris, R.; Jenner, P.; Reynolds, E. H.; Marsden C. D. Clinical, biochemical and physiological features distinguishing myoclonus responsive to 5-hydroxytryptophan, tryptophan with a monoamine oxidase inhibitor, and clonazepam. Brain 100:455–487; 1977.
4. Crespi, F.; Keane, P. E.; Morre, M. Anaesthesia abolishes the effect of valproate on extracellular 5-HIAA, DOPAC and ascorbate as measured in rat striatum by differential pulse voltammetry. Br. J. Pharmacol. 87:279–283; 1986.
5. De Garavilla, L.; Babbs, C. F.; Tacker, W. A. An experimental circulatory arrest model in the rat to evaluate calcium antagonists in cerebral resuscitation. Am. J. Emerg. Med. 2:321–326; 1984.
6. de Lean, J. Action myoclonus and serotonergic activity. N. Engl. J. Med. 296:1414–1415; 1977.
7. de Lean, J.; Richardson, J. C.; Hornykiewicz, O. Beneficial effects of serotonin precursors in postanoxic action myoclonus. Neurology 26:863–868; 1976.
8. Fahn, S. Posthypoxic action myoclonus: Literature review and update. Adv. Neurol. 43:157–169; 1986.
9. Fahn, S. Posthypoxic action myoclonus: Review of the literature and report of two new cases with response to valproate and estrogen. Adv. Neurol. 26:49–84; 1979.
10. Feldman, R. S.; Quenzer, L. F. Fundamentals of neuropharmacology. Sunderland: Sinauer Associates; 1984.
11. Gimenez-Roldan, S.; Mateo, D.; Muradas, V.; De Yebenes, J. G. Clinical, biochemical, and pharmacological observation in a patient with postasphyxic myoclonus: Association to serotonin hyperactivity. Clin. Neuropharmacol. 11:151–160; 1988.
12. Guilleminault, C.; Tharp, B. R.; Cousin, D. HVA and 5-HIAA

CSF measurements and 5-HTP trials in some patients with involuntary movements. J. Neurol. Sci. 18:435–441; 1973.
13. Hallett, M.; Chadwick, D.; Marsden, C. D. Cortical reflex myoclonus. Neurology 29:1107–1125; 1979.
14. Harik, S. I.; Yoshida, S.; Busto, R.; Ginsberg, M. D. Monoamine neurotransmitters in diffuse reversible forebrain ischemia and early recirculation. Neurology 36:971–976; 1986.
15. Hauw, J. J.; Escourolle, R.; Baulac, M.; Morel-Maroger, A.; Goulon, M.; Castaigne, P. Postmortem studies on posthypoxic and postmethyl bromide intoxication: Case reports. Adv. Neurol. 43:201–214; 1986.
16. Hays, W. L. Statistics. New York: CBS Publishing; 1981.
17. Hoyer, D.; Pazos, A.; Probst, A.; Palacios, J. M. Serotonin receptors in the human brain. II. Characterization and autoradiographic localization of 5-HT_{1C} and 5-HT_2 recognition sites. Brain Res. 376:97–107; 1986.
18. Hussong, M. J.; Jaw, S. P.; Aziz, N.; Truong, D. D.; Matsumoto, R. R. Relationship between indole function and a cardiac arrest-induced motor disorder. FASEB J. 8:3789; 1994.
19. Ikarashi, Y.; Sasahara, T.; Maruyama, Y. Postmortem changes in catecholamines, indoleamines, and their metabolites in rat brain regions: Prevention with 10-kW microwave irradiation. J. Neurochem. 45:935–939; 1985.
20. Jaw, S. P.; Hussong, M. J.; Matsumoto, R. R.; Truong, D. D. Involvement of 5-HT_2 receptors in posthypoxic stimulus-sensitive myoclonus in rats. Pharmacol. Biochem. Behav. 49(1):129–131; 1994.
21. Keppel, G. Design and analysis. Englewood Cliffs: Prentice Hall; 1982.
22. Kilpatrick, G. J.; Jones, B. J.; Tyers, M. B. Identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding. Nature 330:746–748; 1987.
23. Lance, J. W.; Adams, R. D. The syndrome of intention or action myoclonus as a sequel to hypoxic encephalopathy. Brain 86:111–136; 1963.
24. L'hermitte, F.; Peterfalvi, M.; Marteau, R.; Gazengel, J.; Serdaru, M. Analyse pharmacologique d'un cas de myoclonies d'intention et d'action postanoxiques, Rev. Neurol. 124:21–31; 1971.
25. Lima, L. Region-selective reduction of brain 5-HT turnover rate and serotonin agonist-induced behavior in mice treated with clonazepam. Pharmacol. Biochem. Behav. 39:671–676; 1991.
26. Matsumoto, M.; Kimura, K.; Fujisawa, A.; Matsuyama, T.; Fukunaga, R.; Yoneda, S.; Wada, H.; Abe, H. Differential effect of cerebral ischemia on monoamine content of discrete brain regions of the mongolian gerbil (*Meriones unguiculatus*). J. Neurochem. 42:647–651; 1984.
27. Patel, S.; Slater, P. Analysis of the brain regions involved in myoclonus produced by intracerebral picrotoxin. Neuroscience 20:687–693; 1987.
28. Pazos, A.; Cotes, R.; Palacios, J. M. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. Brain Res. 346:231–249; 1985.
29. Pranzatelli, M. R.; Snodgrass, S. R. The pharmacology of myoclonus. Clin. Neuropharmacol. 8:99–130; 1985.
30. Pratt, J. A.; Jenner, P.; Marsden, C. D. Comparison of the effects of benzodiazepines and other anticonvulsant drugs on synthesis and utilization of 5-HT in mouse brain. Neuropharmacology 24:59–68; 1985.
31. Schwartz, P. H. An adult and a neonatal model of cerebral ischemia: Participation of excitotoxic mechanisms. Ph.D. Thesis, Los Angeles: UCLA; 1992.
32. Shibasaki, H.; Yamashita, Y.; Kuroiwa, Y. Electroencephalographic studies of myoclonus. Brain 101:447–460; 1978.
33. Slater, P.; Dickinson, S. L. Role of acetylcholine and dopamine in myoclonus induced by intrastriatal picrotoxin. Neurosci. Lett. 28:253–257; 1982.
34. Smirne, S.; Franceschi, M.; Truci, G.; Camerlingo, M.; Pirola, R.; Ferini-Strambi, L.; Bareggi, S. R. Homovanillic acid and 5-hydroxyindoleacetic acid modifications in CSF of patients with stroke and multi-infarct dementia. Stroke 16:1003–1006; 1985.
35. Thal, L. J.; Sparpless, N. S.; Wolfson, L.; Katzman, R. Treatment of myoclonus with L-5-hydroxytryptophan and carbidopa: Clinical, electrophysiological and biochemical observations. Ann. Neurol. 7:570–576; 1980.
36. Truong, D. D.; Matsumoto, R. R.; Schwartz, P. H.; Hussong, M. J.; Wasterlain, C. G. A novel rat cardiac arrest model of posthypoxic myoclonus. Mov. Disord. 9:201–206; 1994.
37. Van Woert, M. H.; Rosenbaum, D.; Chung, E. Biochemistry and therapeutics of posthypoxic myoclonus. Adv. Neurol. 43:171–181; 1986.
38. Van Woert, M. H.; Rosenbaum, D.; Howieson, J.; Bowers, M. B. Long term therapy of myoclonus and other neurological disorders with L-5-hydroxytryptophan and carbidopa. N. Engl. J. Med. 296:70–75; 1977.
39. Wagner, H. R.; Reches, A.; Yablonskaya, E.; Fahn, S. Clonazepam-induced upregulation of serotonin$_1$ and serotonin$_2$ binding sites in rat frontal cortex. Adv. Neurol. 43:645–651; 1986.
40. Wolf, W. A.; Youdim, M. B. H.; Kuhn, D. M. Does brain 5-HIAA indicate serotonin release or monoamine oxidase activity? Eur. J. Pharmacol. 109:381–387; 1985.