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Chronic Treatments With 5-HT_{1A} Agonists Attenuate Posthypoxic Myoclonus in Rats

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JAW, S. P., T. DANG AND D. D. TRUONG. *Chronic treatments with 5-HT_{1A} agonists attenuate posthypoxic myoclonus in rats.* PHARMACOL BIOCHEM BEHAV 52(3) 577–580, 1995.—Following 10 min cardiac arrest and resuscitation, male Sprague–Dawley rats developed posthypoxic myoclonus. This phenomenon peaked at 14 days and disappeared by 60 days after cardiac arrest. From previous results, the 5-hydroxytryptamine (5-HT) system was implicated in the pathogenesis of the disease. In the present study, we investigated the involvement of 5-HT_{1A} receptors in posthypoxic myoclonus in rats. Single injections of 5-HT_{1A} agonists, buspirone (5 and 10 mg/kg body wt.) or 8-OH-DPAT (1, 2, and 4 mg/kg), had no effect on either the intensity or time course of the disease. In contrast, multiple injections (twice a day for 7 or more days) of buspirone (10 mg/kg) or 8-OH-DPAT (4 mg/kg) significantly attenuated the myoclonus scores of animals ($p < 0.05$). The results indicate that chronic stimulation of 5-HT_{1A} receptors in the brain may accelerate endogenous compensatory mechanisms and shorten the time course of the disease.

Hypoxia Ischemia 5-HT_{1A} receptors Posthypoxic myoclonus

MYOCLONUS is defined as sudden, lightening jerks due to involuntary contractions of a muscle or muscle groups (3). Posthypoxic myoclonus (Lance–Adams syndrome) was first described by Lance and Adams (8). Brain levels of serotonin (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 (13). 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. (12). Symptoms of audiogenic myoclonus were relieved in these animals when treated with 5-HTP, valproic acid, and clonazepam; 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.

Questions then arise as to which 5-HT receptor subtype(s) mediate the observed effects. Because 5-HT_{1A} agonists have been demonstrated to have neuroprotective effects on focal and global ischemia (10,11), in this study we investigated the involvement of 5-HT_{1A} receptors in posthypoxic myoclonus in rats.

In the method described by Truong et al. (12), cardiac arrest was induced in rats electrophysiologically via an intracardial injection of KCl to depolarize the heart muscle cells. We introduced an alternative model in which cardiac arrest was mechanically induced by clamping the aorta with an L-shaped loop inserted into the thorax between the second and third ribs. Animals have a better survival rate with the new method than with the prior one. The new method does not have the problems inherent with the old method, such as trauma to the heart by puncturing through the right ventricle, complications caused by high concentration of KCl (e.g., saturating the sodium–potassium exchange pumps leading to renal function failure and so forth). Furthermore, rats treated with the mechanical method responded to classical antimyoclonus drugs such as 5-HTP, valproic acid, and clonazepam in the current studies. We therefore carried out the drug study using the new method.

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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.

Cardiac Arrest and Resuscitation Procedures

We modified the procedures for cardiac arrest described by Truong et al. (12) and Kawai et al. (7). 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 volume. The 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 clamping the arota with an L-shaped loop inserted into the thorax between the second and third ribs and turning off the ventilator. Resuscitation was started 10 min after the arrest by turning on the ventilator (100% O_2, 100 strokes/min), manually compressing the animal's chest, and injecting epinephrine (10 mg/kg) and sodium bicarbonate (4 mEq/kg) IV. Rats were gradually weaned from the ventilator over 2–4 h, the wounds were sutured, and the catheters were 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.

Drug Administration Schedule

For pharmacologic characterization of the mechanical model, 3- to 7-day posthypoxic rats received IP administration of 5-HTP (50 and 100 mg/kg body wt; $n = 6$) (Sigma, St. Louis, MO), clonazepam (0.5 or 1 mg/kg; $n = 6$) (Sigma), valproic acid (50 and 300 mg/kg; $n = 6$) (Sigma), or phenytoin (10 mg/kg; $n = 6$) (Aldrich Chemical Company, Inc., Milwaukee, WI). Myoclonus scores were assessed 30 min before (basal scores) and 60 min after drug administration.

For the acute drug study, 3- to 7-day posthypoxic rats ($n = 12$) were randomly divided into two groups: One group ($n = 6$) received IP administration of buspirone (5 or 10 mg/kg) (RBI, Natick, MA), whereas the other group ($n = 6$) received IP administration of 8-OH-DPAT (1, 2, or 4 mg/kg) (RBI). Myoclonus scores were assessed 30 min before (basal scores) and 60 min after drug administration.

For the chronic drug study, 3-day posthypoxic rats ($n = 18$) were randomly divided into three groups: One group ($n = 6$) received IP administration of buspirone (10 mg/kg), the second group ($n = 6$) received IP administration of 8-OH-DPAT (4 mg/kg), and the third group ($n = 6$) received saline. Injections were given twice a day (1000 and 1600 h). Myoclonus scores were assessed 60 min after drug or saline administration.

Statistics

Changes in myoclonus scores were analyzed by either paired two-tailed Student's t-test or one-way analyses of variance (ANOVA) followed by Dunnett's t-tests. $p < 0.05$ was considered to be significant.

RESULTS

Pharmacological Characterization of the Mechanical Model

Treatments with either 5-HTP (50 or 100 mg/kg), clonazepam (0.5 or 1 mg/kg), or valproic acid (50 or 300 mg/kg) significantly attenuated myoclonus scores of posthypoxic rats (Table 1). In contrast, treatment with phenytoin (10 mg/kg) had no significant effect on the expression of posthypoxic myoclonus in rats (Table 1).

Drug Study

A single injection of either buspirone (5 or 10 mg/kg) or 8-OH-DPAT (1, 2, or 4 mg/kg) did not affect myoclonus scores significantly in posthypoxic rats (Table 1). In contrast, multiple injections of buspirone (10 mg/kg, twice a day for 7 days) significantly attenuated myoclonus scores of animals (Table 2). Similar results were found in posthypoxic rats that received multiple injections of 8-OH-DPAT (4 mg/kg) twice a day for 7 days (Table 2). Finally, posthypoxic rats receiving saline vehicle injections developed myoclonus upon audiogenic stimulation (Table 2).

TABLE 1

EFFECTS OF SINGLE INJECTIONS OF DRUGS ON MYOCLONUS SCORES (PERCENT OF CONTROL VALUES) OF POSTHYPOXIC RATS

Drugs	Doses (mg/kg)	Basal	Treatment
5-HTP	50	100 ± 4*	72 ± 3†
	100	100 ± 3	66 ± 5†
Clonazepam	0.5	100 ± 4	79 ± 4†
	1	100 ± 5	68 ± 6†
Valproic acid	50	100 ± 4	84 ± 5†
	300	100 ± 3	76 ± 4†
Phenytoin	10	100 ± 2	97 ± 3
8-OH-DPAT	1	100 ± 6	97 ± 3
	2	100 ± 4	98 ± 6
	4	100 ± 5	106 ± 4
Buspirone	5	100 ± 4	102 ± 3
	10	100 ± 5	105 ± 6

*Values are mean ± SEM from six rats before and after IP administration of drugs.

†$p < 0.05$; values are significantly different from those of control values as determined by paired two-tailed Student's t-test.

TABLE 2

EFFECTS OF MULTIPLE INJECTIONS OF 5-HT$_{1A}$ AGONISTS ON MYOCLONUS SCORES OF POSTHYPOXIC RATS

Treatment*	Myoclonus Scores
Saline	184 ± 7†
Buspirone	123 ± 6‡
8-OH-DPAT	114 ± 5†‡

*Rats received IP injections of saline, buspirone (10 mg/kg), or 8-OH-DPAT (4 mg/kg) twice a day for 7 days.

†Values are mean ± SEM from six rats.

‡$p < 0.05$; values are significantly different from those of control values as determined by ANOVA followed by Dunnett's t-test.

DISCUSSION

Pharmacological Characterization of the Mechanical Model

Rats from this model responded to classical antimyoclonus drugs (e.g., 5-HTP, clonazepam, and valproic acid), but not to the anticonvulsant phenytoin. The jerking movements observed were not seizures, because animals maintained upright postures and the ability to right themselves. Furthermore, the movements, unlike seizures, were time-locked to the audiogenic stimuli. These movements were also not startle responses, because the metronome (rise time: 15 ms; intensity: 95 dB) used in the present study was different from that used to produce a startle response (rise time: <12 ms; intensity: 115 dB). When the startle response was elicited and recorded in posthypoxic rats using an automated startle device (SR-LAB Startle Reflex System; San Diego Instruments, San Diego, CA), it was found that there was no correlation between scores of myoclonus and the startle response of the same rats. In addition, clonazepam and valproic acid attenuated myoclonus scores but exerted no effect on startle response scores. Based on these observations as well as those discussed in Truong et al. (12), we believe that the current mechanical model is a valid model for the study of posthypoxic myoclonus.

The finding that myoclonic rats responded to two antimyoclonic/anticonvulsant drugs (e.g., clonazepam, valproic acid) suggests that myoclonus and seizure may use a common neuronal pathway to generate the symptomatology. That is to say, myoclonus may reflect a part of the seizure formation (a mild form of seizure). It is possible that myoclonus and seizure only reflect differences in the magnitude of activation or recruitment of some common neuronal pathway. On the other hand, the finding that myoclonic rats did not respond to other anticonvulsant drug, phenytoin, suggests that myoclonus may possess its own unique generational circuitry with only some parts overlapping with the pathway that generates seizure. This may explain why some anticonvulsants may work as antimyoclonics, whereas others do not.

General Hypotheses

We propose that posthypoxic myoclonus may be due to either decreases in inhibitory tones (disinhibition), excessive activities in the excitatory circuitry, or both, of the CNS following hypoxic-ischemic insults. Furthermore, stimulus-sensitive myoclonus may result from a mismatch between the sensory apparatus and the motor sytem of the brain, leading to exaggerated responses upon stimulation.

In our studies as well as in those described by Truong et al. (12), rats developed posthypoxic myoclonus only after specific damage occurred to the brain following hypoxic-ischemic insults. For example, after 1 min cardiac arrest and resuscitation, rats appeared to be normal after recovery. However, rats that recovered from 4 min cardiac arrest developed seizure, and most (75%) died afterward. Only after 10 min cardiac arrest did animals develop stimulus-sensitive myoclonus. These phenomena may be because 4 min hypoxia-ischemia damaged the inhibitory system of the brain first; thus, the excitatory neuronal circuitry generating seizure went unchecked and rats died subsequently from overexcitation of the brain. Furthermore, longer periods of hypoxia-ischemia (10 min) may damage not only the inhibitory system but also neurons of the excitatory circuitry generating seizure; thus, animals survived with myoclonus. When the state of cardiac arrest lasted longer than 10 min, rats simply never came back from resuscitation; the damage was so great that it reached a point beyond recovery.

Roles of 5-HT$_{1A}$ Receptors in Posthypoxic Myoclonus

A single injection of the 5-HT$_{1A}$ agonist buspirone or 8-OH-DPAT failed to affect the expression of myoclonus in rats, although activation of 5-HT$_{1A}$ receptors is known to inhibit neurons in the hippocampus, cortex, and dorsal Raphe nuclei (9). The results tend to support the disinhibition theory of myoclonus proposed earlier; that is to say, the inhibitory components of the CNS (e.g., 5-HT-ergic neuronal systems) are already compromised by hypoxia-ischemia incidents (3,4, 6,12); thus, inhibitory effects from the activation of 5-HT$_{1A}$ receptor (e.g., presynaptic 5-HT$_{1A}$ receptors on the dorsal Raphe nuclei) would be compromised as well. Therefore, myoclonus scores of posthypoxic rats were not affected by acute treatments of 5-HT$_{1A}$ agonists.

On the other hand, the elevated serotonergic tone may be required to suppress the excitatory circuitry that generates myoclonus in rats upon audiogenous stimulation (3,4,12). This modulation by intact endogenous 5-HT system is present in normal rats, whereas in posthypoxic rats the system is somehow attenuated (3,4,6,12). Therefore, further inhibition of 5-HT neurons in the Raphe nuclei from 5-HT$_{1A}$ receptor activation would not affect the outcome of the disease and might even make it worse by letting the excitatory circuitry generating myoclonus go further unchecked. This may be the reason why acute treatments of 5-HT$_{1A}$ agonists failed to affect myoclonus scores in posthypoxic rats. However, the functional significance of the present results needs to be explored further. In short, the acute treatment results tend to rule out the involvement of 5-HT$_{1A}$ receptors in the pathogenesis of posthypoxic myoclonus in rats.

In contrast, chronic treatments with either buspirone or 8-OH-DPAT significantly attenuated myoclonus scores of animals. Several factors may have contributed to this. First, the evolution of brain damage in global ischemia takes 1–7 days to develop fully (6,7,10,11). Furthermore, neuroprotective treatment needs to be maintained for prolonged periods for efficacy. There is considerable evidence that chronic treatment with 5-HT$_{1A}$ agonists reduces damage after global ischemia (10,11). Alternatively, 5-HT$_{1A}$ agonists can produce hypothermia, which is itself neuroprotective. Finally, chronic stimula-

tion on astrocytic 5-HT_{1A} receptors by agonists may lead to increased synthesis of serotonin growth factor (S100β), which in turn promotes survival and neurite outgrowth of serotonergic neurons (14–17). Once the serotonergic tone in the brain is restored, rats no longer have myoclonus (5,6).

Conclusions

The mechanically induced cardiac arrest rat model is a valid model for the study of posthypoxic myoclonus. The current results tend to further support the hypothesis that hypoactivity of 5-HT neurotransmission may underlie the development of posthypoxic myoclonus. A treatment regimen that includes the chronic administration of 5-HT_{1A} agonists may have added benefits in accelerating endogenous compensatory mechanisms and shorten the time course of the disease for human patients with posthypoxic myoclonus.

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