Pharmacology Biochemistry & Behavior, Vol. 15, pp. 363–366, 1981, Printed in the U.S.A.

Effect of Brain Serotonin Level on Induced Hippocampal Paroxysmal Activity in Rats[1]

ESPER A. CAVALHEIRO, ELAINE ELISABETSKY[2]
AND CARLOS J. R. CAMPOS[3]

Neurofisiologia, Departamento do Fisiologia, Escola Paulista de Medicina
Rua Botucatu, 862, 04023 Sao Paulo, S.P., Brasil

Received 11 February 1980

CAVALHEIRO, E. A., E. ELISABETSKY AND C. J. R. CAMPOS. *Effect of brain serotonin level on induced hippocampal paroxysmal activity in rats.* PHARMAC. BIOCHEM. BEHAV. 15(3) 363–366, 1981.—Experiments were performed to study the involvement of brain 5-HT in an experimental model of epilepsy induced by repeated electrical stimulation of the dorsal hippocampus of rats. The experiments included: (1) systemic injections of 5-hydroxytryptophan (5-HTP) and p-chlorophenylalanine (pCPA) and (2) electrolytic lesions of the midbrain raphe nuclei. The pCPA group showed a significant increase while animals which received systemic injections of 5-HTP showed a great reduction in the electrographic seizure activity. Although several reports have shown that midbrain raphe lesions do not modify the epileptic susceptibility, we observed a clear enhancement in the epileptiform activity in lesioned animals. The results presented here support the view that serotonergic systems may exert a tonic inhibitory effect on hippocampal epileptic activity.

Hippocampus pCPA 5-HTP 5-HT Electrical stimulation Experimental epilepsy
Midbrain raphe lesions

IN the last decade the kindling method of Goddard *et al.* [17] has been used widely and its modifications are considered useful to other laboratory assays for antiepileptic agents [35]. This model has also been used in the study of facilitation and inhibition of epileptic phenomena which involve many central neurotransmitter systems [22]. The basic view underlying this approach is that epileptic phenomena including kindling should be understood as a modification in synaptic conduction [17]. Available evidence suggests that kindled convulsions result from either increased synaptic conduction in excitatory neural systems or decreased synaptic efficiency in inhibitory neural pathways [2,14]. This evidence is mainly supported by data derived from different experimental models of epilepsy where high cerebral concentrations of inhibitory transmitters, norepinephrine, dopamine and serotonin (5-HT) decrease while low cerebral 5-HT and catecholamine levels increase seizure susceptibility [27].

In two experiments reported here we tested the inhibitory role of 5-HT systems on an experimental model of epilepsy introduced by Taber *et al.* [33], where longterm self sustained seizures were produced in mice using an interstimulus interval of one minute via hippocampal electrode placements. We have employed this modification of Goddard's kindling phenomenon using rats as experimental animals [7, 8, 11]. No behavioral differences were noticed between our animals and those described by Taber *et al.* [33]. In the first experiment we report the effect of systemic injections of 5-hydroxytryptophan (5-HTP), which induces an increase of 5-HT synthesis and release [23,24], and p-chlorophenylalanine (pCPA), a 5-HT synthesis inhibitor [15,29] on this experimental model of epilepsy. The other one was carried out to observe the effect of midbrain raphe lesions to destroy the n. raphe median which supplies most of the 5-HT afferents to the hippocampal formation [3, 4, 10, 24].

EXPERIMENT 1—SYSTEMIC 5-HTP AND pCPA

METHOD

Male albino Wistar rats (250–300 g) were used. Under deep anesthesia (ketamine, 60 mg/kg, IM, plus nembutal, 20 mg/kg, IP), bipolar twisted steel wire electrodes (100 μm) were stereotaxically implanted unilaterally into the dorsal hippocampus according to the atlas of De Groot [12] (A=3.0, L=4.0, H=2.8 from the cortex surface) and cemented in place with dental acrylic. The electrodes were insulated with

[1]Supported by research grants from FAPESP (Brasil) and from CNPq (Brasil) to Dr. E. A. Cavalheiro and by an Institutional grant from FINEP (Brasil) to the Escola Paulista de Medicina. The authors thank Dr. G. Le Gal La Salle for his useful discussion.
[2]Fellow from FAPESP, Brasil.
[3]Fellow from CNPq, Brasil.

TABLE 1

EFFECT OF SYSTEMIC pCPA AND 5-HTP ON THE EPILEPTIC ACTIVITY OF RATS SUBMITTED TO REPEATED ELECTRICAL STIMULATION OF THE DORSAL HIPPOCAMPUS

	Group		
	Control (implanted + vehicle)	pCPA	5-HTP
Control Session	29.1 ± 5.91	28.6 ± 5.08	31.5 ± 7.05
Test Session	34.6 ± 4.16	48.8 ± 9.64*	11.0 ± 1.28*

Data (% of EEG time occupied by epileptic activity during the 2 hour session) expressed as mean ± S.D. Ten animals per group.

*Significant to 1% level (Student *t*-Test) when compared with control values at the same session.

enamel except for the cross sections of the cut ends. One week later, animals were connected to a Beckman polygraph through a flexible cable that was also connected to a relay box which automatically selected the animal's electrode connections between the polygraph and the stimulator. Hippocampal EEG was monitored throughout the experimental sessions. Brain stimulation was delivered at one minute intervals. A constant current stimulator delivered 1 msec byphasic square wave pulses at 60 Hz for 1 sec. Current intensity ranged between 300 and 800 μA. The EEG was analysed for the percent afterdischarge (AD) occurring during each one-minute interval for 2 hours (Control Session). After the control session the animals were subdivided in 3 groups: one for pCPA injections, another for 5-HTP and the last as control. pCPA was mixed in several drops of Tween-80 suspended in saline and injected IP at a dose of 100 mg/kg for three consecutive days. The first injection was done after the control session and, 24 hours after the last dose, the animals were submitted to a second stimulation session (Test Session). 5-HTP was also mixed in Tween-80 suspended in saline and administered at a dose of 50 mg/kg, IP, one hour before the test session. Control rats received IP injections of an equal volume of the vehicle alone. All animals received IP injections of the vehicle at the moments in which the test drugs (pCPA or 5-HTP) were injected to the corresponding groups. During the test session the same parameters of stimulation for each animal were used and the quantity of epileptiform activity compared to that observed during the control session.

RESULTS

As previously reported [11] a characteristic electroencephalographic sequence of animals submitted to an intermitent electrical stimulation of the dorsal hippocampus shows periods of neuronal discharge activity generating spikes and/or waves of varying potential. Early stimuli did not produce sustained afterdischarges and not every stimulus induced an AD. As stimulation progressed, the amplitude and duration of each AD increased. The seizure activity was sometimes followed by depression of normal hippocampal activity. No behavioral or autonomic changes occurred when the discharge was of short duration, the animal usually remaining quiet and still. As the duration of the AD increased, the animals showed increased respiration rate, pupillary changes, piloerection, salivation, blinking and chewing, followed by contractions of the head, fore and hind paws.

Table 1 shows the effect of systemic pCPA and 5-HTP on this epileptic activity. As we can see, there is a clear relationship between 5-HTP level and seizure activity. The pCPA treated group showed a marked increase in the percent of AD while in the 5-HTP group we can observe a significant decrease in the AD duration. Behaviorally, pCPA animals showed a greater number of generalized convulsions during the two-hour session than the control group. The 5-HTP treated animals during the test session showed almost only masticatory movements and no animal had a generalized seizure.

The increase in the duration of the epileptic recording observed in the control group during the test session compared to that observed during the control session has been attributed by us to the kindling effect [7].

EXPERIMENT 2—MIDBRAIN RAPHE LESIONS

METHOD

Male albino Wistar rats submitted to the surgical procedure described for Experiment 1, also received electrolytic lesions of the midbrain raphe nuclei (A=−0.4, L=0.0, H=4.5, according to the atlas of Pellegrino and Cushman, [25]). Lesions were made via a steel electrode (125 μm), insulated with enamel except for 0.2 mm at the tip, lowered to the desired position and 1.5 mA DC was passed between the tip and a rectal cathode for 10 sec. In the sham-operated group electrode was placed at the same A and L coordinates as in the lesioned animals, but was lowered only 0.5 mm down from the cortical surface and current was not passed. One control group with implanted animals was also used. Two weeks after surgery the animals were submitted to the two hour stimulating session using the same procedure described for the Experiment 1, during which the epileptic activity was recorded.

The whole brain of half of the animals from the experiments 1 and 2, randomly chosen, was immediately homogenized frozen and used later for fluorometric determination of 5-HT according to Bogdanski *et al.* [5]. The other half was stored in Formalin for later histological analyses (Figs. 1 and 2).

RESULTS

As can be seen in Table 2 there was a significant increase in the electrographic epileptic activity of the hippocampus of the raphe lesioned animals similar to that observed in the animals which received IP injections of pCPA (Experiment 1).

Table 3 shows brain 5-HT levels of animals of Experiments 1 and 2.

GENERAL DISCUSSION

The results reported here show a serotonergic inhibition of hippocampal epileptic activity in rats. Reduction in the 5-HT level induced by pCPA was correlated with augmented epileptiform activity in the hippocampus. Conversely, this effect was greatly reduced when the animals were treated with 5-HTP.

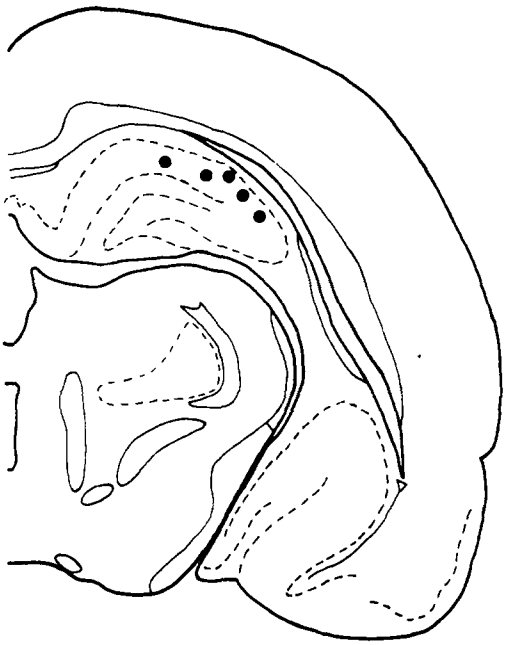

FIG. 1. Locations of electrode tips in the dorsal hippocampus of rats. The brain section depicted here corresponds, approximately, to the plane A=3.0 from the atlas of De Groot [12].

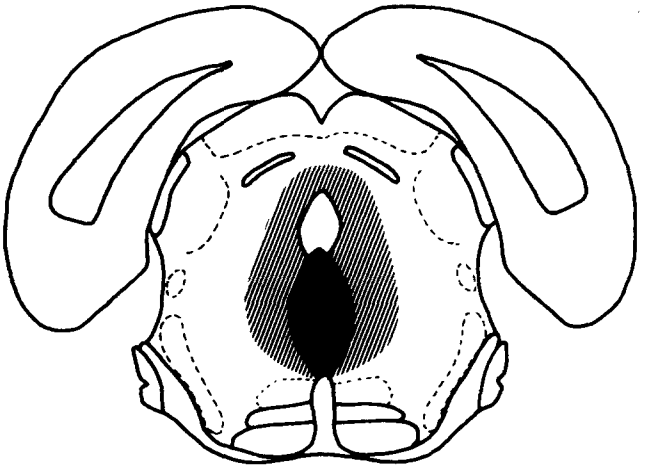

FIG. 2. Maximum (striped) and minimum (darkened) extent of midbrain raphe lesions. Plane A=−0.4 from the atlas of Pellegrino and Cushman [25].

TABLE 2

EFFECT OF MIDBRAIN RAPHE LESION ON EPILEPTIC ACTIVITY OF RATS SUBMITTED TO REPEATED ELECTRICAL STIMULATION OF THE DORSAL HIPPOCAMPUS

	Implanted control	Sham-operated group	Midbrain raphe lesions group
% Epileptic activity	27.8 ± 3.54	30.5 ± 5.37	43.1 ± 4.94*

Data (as in Table 1) expressed as mean ± S.D. Ten animals per group.

*Significant to 1% level (Student *t*-Test) when compared to both control values.

Raphe lesioned animals also showed a significant increase in the electrographic epileptic activity. Although the effect of midbrain raphe lesions is in agreement with the effect of systemic injections of pCPA several reports show that midbrain raphe lesions do not influence ECS threshold [26] or severity [6]. A possible explanation for these discrepant results could be based on the fact that convulsive seizures induced by different methods do not necessarily share the same basic mechanisms. Furthermore, in our experiments we utilized direct hippocampal stimulation to induce epileptic activity which could be a more sensitive preparation to study the role of a specific neurotransmitter system. We could thus demonstrate the lack of the 5-HT inhibition following raphe lesions.

Results obtained through other experimental models of epilepsy, as for example, audiogenic seizures [19,30], pentylenetetrazol induced seizures [1,13], ECS induced convulsions [9,21], cortical or amygdaloid kindling [28,32], support the hypothesis that 5-HT systems inhibit seizure activity.

The results of the present study suggest that 5-HT is necessary for the regulation of hippocampal excitability. Recently, Segal [31] reported that both electrical stimulation in the raphe nuclei and 5-HT applied iontophoretically in the

TABLE 3

BRAIN 5-HT CONTENT (μg/g) OF RATS WHICH RECEIVED SYSTEMIC pCPA OR 5-HTP AND WITH MIDBRAIN RAPHE LESIONS

	Control	Sham-operated animals	pCPA	5-HTP	Midbrain raphe lesions
Brain 5-HT content	0.47 ± 0.04 (10)	0.43 ± 0.05 (5)	0.23 ± 0.03* (5)	1.46 ± 0.06† (5)	0.26 ± 0.02* (5)

Data expressed as mean ± S.D.; n in parentheses.

Significant to *5% and †1% levels (Student *t*-Test) when compared with control and sham-operated groups.

Control value represents the mean of control animals injected with vehicle alone in Experiment 1 and implanted controls in Experiment 2.

hippocampus decrease hippocampal cell firing frequency, while Jahnsen [18] indicated that a function of 5-HT in the hippocampus may be to inhibit dendritic excitatory synapses, which could explain our findings.

However, alterations of serotonergic mechanisms constitute probably only one of many neurochemical alterations occurring during epileptic activity. Indeed, Meldrum [22] in a review, reported many papers relating the involvement of other neurotransmitters in the epileptic phenomenon and we should not forget important neurotransmitter systems interactions [16,20].

REFERENCES

1. Alexander, G. J. and L. M. Kopeloff. Metrazol seizures in rats: effects of p-chlorophenylalanine. *Brain Res.* **22:** 231–235, 1970.
2. Arnold, P., R. Racine and R. Wise. Effects of atropine, reserpine, 6-hydroxydopamine, and handling on seizure development in the rat. *Expl Neurol.* **40:** 457–470, 1973.
3. Azmitia, E. C. and M. Segal. An autoradiographic analysis of the different ascending projections of the dorsal and median raphe nuclei in the rat. *J. comp. Neurol.* **179:** 641–668, 1978.
4. Bobiller, P., S. Seguin, A. Degueurce, B. D. Lewis and J. F. Pujol. The efferent connections of the nucleus raphe centralis superior in the rat as revealed by radioautography. *Brain Res.* **166:** 1–8, 1979.
5. Bogdanski, D. F., A. Pletscher, B. B. Brodie and S. Udenfriend. Identification and assay of serotonin in brain. *J. Pharmac. exp. Ther.* **117:** 82–88, 1956.
6. Browning, R. A., W. E. Hoffman and R. L. Simonton. Changes in seizure susceptibility after intracerebral treatment with 5,7-dihydroxytryptamine: the role of serotonergic neurons. *Ann. N. Y. Acad. Sci.* **305:** 437–456, 1978.
7. Campos, C. J. R. and E. A. Cavalheiro. Modificaçao do método kindling para obtençao de status epilepticus experimental em ratos. *Archos Neuropsiquiat., S Paulo* **38:** 81–88, 1980.
8. Campos, C. J. R. and E. A. Cavalheiro. The paradoxical effect of lidocaine on an experimental model of epilepsy. *Archs int. Pharmacodyn.* **243:** 66–73, 1980.
9. Chen, G., C. R. Ensor and B. Bohner. Drug effects on the disposition of active biogenic amines in the CNS. *Life Sci.* **7:** 1063–1074, 1968.
10. Conrad, L. C. A., C. M. Leonard and D. W. Pfaff. Connections of the medial and dorsal raphe nuclei in the rat: an autoradiographic and degeneration study. *J. comp. Neurol.* **156:** 179–206, 1974.
11. Cowan, C. M. and E. A. Cavalheiro. Epilepsy and Membrane Na^+K^+ ATPase: Changes in activity using an experimental model of epilepsy. *Acta physiol. latinoamer.* **30:** 253–258, 1980.
12. De Groot, J. The rat forebrain in stereotaxic coordinates. *Proc. K. ned. Akad. Wet.* (C) **52:** 1–40, 1959.
13. De la Torre, J. C., H. M. Kawanaga and S. Mullen. Seizure susceptibility after manipulation of brain serotonin. *Archs int. Pharmacodyn.* **188:** 298–304, 1970.
14. Drucker-Colin, R. Seizure inhibition and REM sleep: a possible neurohumoral relationship. *Behav. Biol.* **12:** 199–209, 1974.
15. Fuller, R. W. Pharmacology of central serotonin neurons. *A. Rev. Pharmac. Toxicol.* **20:** 111–127, 1980.
16. Garcia Ramos, J. and E. de la Cerda. Cortical effects of the electrical stimulation of the Locus coeruleus. *Acta physiol. latinoamer.* **25:** 311–320, 1977.
17. Goddard, C. V., D. C. McIntyre and C. K. Leech. A permanent change in brain function resulting from daily electrical stimulation. *Expl Neurol.* **25:** 295–330, 1969.
18. Jahnsen, H. The action of 5-hydroxytryptamine on neuronal membranes and synaptic transmission in area CA1 of the hippocampus in vitro. *Brain Res.* **197:** 83–94, 1980.
19. Jobe, P., A. Picchioni and L. Chin. Role of brain 5-hydroxytryptamine in audiogenic seizures in the rat. *Life Sci.* **13:** 1–13, 1973.
20. Johnson, G. A., E. G. Kim and S. J. Boukma. Mechanism of norepinephrine depletion by 5-hydroxytryptophan. *Proc. Soc. exp. Biol. Med.* **128:** 509–512, 1968.
21. Koe, B. K. and A. Weissman. The pharmacology of parachlorophenylalanine, a selective depletor of serotonin stores. *Adv. Pharmac.* **68:** 29–47, 1968.
22. Meldrum, B. S. Neurotransmitters and Epilepsy. In: *Neurotransmitter Systems and Their Clinical Disorders*, edited by N. J. Legg. New York: Academic Press, 1978, pp. 167–181.
23. Moir, A. T. B. and D. J. Eccleston. The effect of precursor loading on the cerebral metabolism of 5-hydroxyindoles. *J. Neurochem.* **15:** 1093–1108, 1968.
24. Moore, R. Y., A. E. Halaris and B. E. Jones. Serotonin neurons of midbrain raphe: ascending projections. *J. comp. Neurol.* **180:** 417–438, 1978.
25. Pellegrino, L. J. and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain.* New York: Appleton Century-Crofts, 1967.
26. Quattrone, A., V. Crunelli and R. Samanin. Seizure susceptibility and anticonvulsant activity of carbamazepine, diphenylhydantoin and phenobarbital in rats with selective depletions of brain monoamines. *Neuropharmacology* **17:** 643–647, 1978.
27. Racine, R. Kindling: The first decade. *Neurosurgery* **3:** 234–252, 1978.
28. Racine, R. and D. V. Coscina. Effects of midbrain raphe lesions or systemic p-chlorophenylalanine on the development of kindled seizures in rats. *Brain Res. Bull.* **4:** 1–7, 1979.
29. Sanders-Bush, E. and V. J. Massari. Actions of drugs that deplete serotonin. *Fedn Proc.* **36:** 2149–2153, 1977.
30. Schlesinger, K., W. Boggan and D. K. Freedman. Genetics of audiogenic seizures. II. Effects of pharmacological manipulation of brain serotonin, norepinephrine and gamma-aminobutyric acid. *Life Sci.* **7:** 437–447, 1968.
31. Segal, M. Physiological and pharmacological evidence for a serotonergic projection to the hippocampus. *Brain Res.* **94:** 115–131, 1975.
32. Siegel, J. and G. J. Murphy. Serotonergic inhibition of amygdala-kindled seizures in cats. *Brain Res.* **174:** 337–340, 1979.
33. Taber, K. H., J. J. McNamara and S. F. Zornetzer. Status epilepticus: a new rodent model. *Electroenceph. clin. Neurophysiol.* **43:** 707–729, 1977.
34. Ternaux, J. P., A. Boireau, S. Bourgoin, M. Hamon, F. Hery and J. Glowinski. In vivo release of 5-HT in the lateral ventricle of the rat: Effects of 5-hydroxytryptophan and tryptophan. *Brain Res.* **101:** 533–548, 1976.
35. Wada, J. A. Pharmacological prophylaxis in the kindling model of epilepsy. *Archs Neurol.* **34:** 389–395, 1977.