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Comparison of Regional Brain 5-HT and 5-HIAA Content in Flexor and Extensor Rats

R. A. BROWNING, J. K. SMITH AND M. T. BRANDON

Department of Physiology and Pharmacology, Southern Illinois University School of Medicine Carbondale, IL 62901

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BROWNING, R. A., J. K. SMITH AND M. T. BRANDON. *Comparison of regional brain 5-HT and 5-HIAA content in flexor and extensor rats.* PHARMACOL BIOCHEM BEHAV **18**(4) 525–528, 1983.—Hindlimb extension (HLE) induced by maximal electroshock seizures (MES) can be markedly affected by drugs which affect CNS 5-hydroxytryptamine (5-HT). Consequently, it has been proposed that the natural resistance of certain rats (flexor rats) to HLE is due to elevated levels of 5-HT. We have tested the hypothesis that the increased resistance of flexor rats to MES-induced HLE is due to elevated serotonergic levels in some region(s) of the CNS by examining 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels in 8 regions of the CNS in rats classified by MES as either flexors or extensors. Furthermore, we compared the *in vivo* synthesis rate of 5-HT between flexor and extensor rats in 6 regions of the brain by measuring the accumulation of 5-HTP following aromatic amino acid decarboxylase inhibition with NSD-1015. All neurochemical analyses were carried out on rats sacrificed one week after their last seizure test. No differences in 5-HT, 5-HIAA or 5-HTP synthesis rate were detected between flexor and extensor rats for any of the regions examined, suggesting that enhanced serotonergic levels are not responsible for the unusual resistance of flexor rats to HLE.

5-HT 5-HIAA 5-HTP Flexor rats Extensor rats Hindlimb extension (HLE) Maximal electroshock seizures (MES)

RATS may be classified by maximal electroshock as "flexors" if they fail to exhibit hindlimb extension (HLE) or "extensors" if they consistently exhibit HLE. Only a small percentage (8–10%) of male Sprague-Dawley rats are consistent flexor rats. However, based on the work of Zablocka and Esplin [18] and more recently the studies of Buterbaugh [4,5] these animals appear to provide an interesting animal model for studying mechanisms of natural resistance to tonic seizures. Since abolition of HLE in the maximal electroshock seizure (MES) is widely used as an index of anticonvulsant drug activity, it is of interest to know more about the natural mechanisms that regulate HLE. Buterbaugh [4,5] has shown that the MES-induced HLE is quite sensitive to manipulations in brain 5-hydroxytryptamine (5-HT/serotonin). Thus, flexor rats can be converted to extensor rats by depletion of 5-HT, while extensor rats are converted to flexor rats by treatments that increase 5-HT receptor activation. Moreover, it has been suggested that the resistance of flexor rats to HLE is due to increased whole brain levels of 5-HT [4]. On the other hand, manipulations in brain norepinephrine (NE), which affect most other seizure models [12], do not appear to affect the flexor rat [5]. The present studies were therefore undertaken to test further the hypothesis that elevated levels of serotonin are associated with the resistance of flexor rats to MES, and to gain a better understanding of where 5-HT might be acting in the CNS to inhibit HLE. Since Buterbaugh [4] had examined whole brain 5-HT, we elected to compare regional levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) between flexor and extensor rats. The *in vivo* synthesis rate of 5-HT in various regions of rat brain were also compared between flexor and extensor rats.

METHOD

Animals

Male Sprague-Dawley rats (250–275 g) were obtained from Harlan-Sprague-Dawley, Madison, WI. They were maintained on a 12 hr light-dark cycle and housed 3 per cage with food and water ad lib.

Maximal Electroshock Seizure (MES) Test

Prior to neurochemical analysis, rats were classified as either flexors or extensors according to their response in the MES test. Each rat was subjected to 3 maximal seizures with a 48 hour interval between successive tests. Seizures were produced by stimulation with 150 mA (60 Hz AC) through corneal electrodes for 200 msec using the apparatus described by Woodbury and Davenport [17]. Animals exhibiting flexion without evidence of HLE on all 3 occasions were classified as flexors, while those showing full HLE in all 3 tests were classified as extensors. Rats which failed to show a consistent response were eliminated from further study.

5-HT, 5-HIAA Analysis

Because brain 5-HT receptors and turnover can be altered for up to 48 hours after an electroshock seizure [8, 10, 11] the

TABLE 1

REGIONAL 5-HT AND 5-HIAA IN FLEXOR AND EXTENSOR RATS*

CNS Region	5-HT ng/g		5-HIAA ng/g	
	Flexors	Extensors	Flexors	Extensors
Cortex	296.8 ± 19 (6)	300.1 ± 18 (6)	252.8 ± 14 (6)	297.0 ± 20 (6)
Hippocampus	473.5 ± 20 (5)	480.8 ± 15 (6)	626.0 ± 64 (5)	668.7 ± 48 (6)
Hypothalamus	918.6 ± 59 (7)	909.6 ± 62 (6)	703.2 ± 120 (7)	676.7 ± 45 (6)
Midbrain	1022.5 ± 35 (7)	981.8 ± 43 (6)	1239.7 ± 49 (7)	1237.1 ± 57 (5)
Cerebellum	111.7 ± 6 (7)	117.5 ± 12 (7)	213.8 ± 1 (7)	221.5 ± 6 (7)
Pons-Medulla	552.3 ± 21 (7)	580.1 ± 29 (5)	615.9 ± 36 (7)	657.4 ± 28 (5)
Cervicothoracic Cord	209.8 ± 7 (7)	224.1 ± 23 (7)	389.6 ± 9 (7)	358.1 ± 20 (7)
Lumbar Cord	548.4 ± 25 (7)	498.6 ± 27 (7)	580.5 ± 29 (7)	563.2 ± 36 (7)

*Rats were sacrificed 1 week after their last seizure test. Numbers in parentheses indicate the number of rats in each group. No significant differences were detected between flexor and extensor rats using two-tailed *t*-test.

animals were allowed to rest for one week following their last seizure to avoid any lingering effects on neurochemical measurements. One week after their last MES test, rats were sacrificed by decapitation and the brains were quickly removed. An initial cut was made through the optic chiasm and the hemisphere overlying the diencephalon and midbrain was peeled forward and separated from the rest. The hippocampus and amygdala were dissected away from the peeled portion, resulting in the hippocampal, and cortical samples. The hypothalamus was obtained by making a coronal cut at the caudal border of the mammillary body and a horizontal cut through the anterior commissure. The midbrain was then separated from the pons-medulla by a coronal cut at the rostral border of the pons. The pons-medulla consisted of the area between the rostral border of the pons and the obex. The cervicothoracic spinal cord was separated from the lumbar cord by a cut at the level of the 13th thoracic vertebrae just below the end of the rib cage. During dissection of the brain, the spinal cord was being removed by a separate investigator. All dissections were carried out rapidly at room temperature and the time between decapitation and freezing was less than 3 min for brain regions and less than 4 min for spinal cord. Following dissection the samples were immediately frozen in liquid nitrogen where they remained until assay. All assays were performed within 3 weeks after the animals were killed.

5-Hydroxytryptophan (5-HTP), 5-HT and 5-HIAA were assayed by high performance liquid chromatography (HPLC) according to a modification of the procedure described by Perry and Fuller [14]. Samples were homogenized in 5 ml of acidified butanol (containing 0.85 ml concentrated HCl per liter) and centrifuged to pellet the tissue (2500 RPM, IEC Model K). Four ml of the supernatant were transferred to tubes containing 4 ml of heptane and 0.5 ml of 0.2 M phosphate buffer (pH 7) containing 500 μg/ml of cysteine. After shaking for 20 min and centrifuging, 50 μl of the phosphate layer was injected into a Bioanalytical HPLC system equipped with a Biophase (5 μ) column and a glassy carbon electrode. The mobile phase consisted of 0.1 M sodium acetate containing 6% methanol with a pH of 4.7. The flow rate was maintained at 2 ml per min. Standards containing 10, 20 or 40 ng of 5-HTP, 5-HT and 5-HIAA (free base) in 20 μl of 0.01 N HCl were added to separate homogenizers containing 5 ml of acidified butanol and carried through the entire extraction to correct for recovery and for the calculation of tissue concentration.

Whole brain 5-HT levels were also measured in flexor and extensor rats using the fluorometric procedure of Bogdanski *et al.* [1].

In Vivo *Tryptophan Hydroxylase Activity*

The *in vivo* tryptophan hydroxylase activity was determined by inhibiting aromatic L-amino acid decarboxylase activity with NSD-1015 (3-hydroxybenzyl hydrazine HCl) and measuring the accumulation of 5-HTP according to the method of Carlsson *et al.* [6]. Rats were given NSD-1015 (100 mg/kg, IP) and sacrificed 30 min later. To obtain the zero time point, saline instead of NSD-1015 was administered. The amount of 5-HTP accumulated in 30 min was then determined using the same HPLC assay described above.

Data

All values reported in tables are expressed as the mean plus or minus the standard error. Comparisons between flexor and extensor rats were made using a two-tailed student's *t*-test.

RESULTS

The mean 5-HT and 5-HIAA concentrations for cortex,

hippocampus, hypothalamus, midbrain, cerebellum, pons-medulla, cervicothoracic spinal cord, and lumbar spinal cord are presented in Table 1. As can be seen, no significant differences were detected between flexor and extensor rats. Inasmuch as 5-HT turnover rate is more indicative of the activity of 5-HT neurons than steady-state levels, we examined the *in vivo* activity of tryptophan hydroxylase in various brain regions of flexor and extensor rats. Table 2 shows that there were no significant differences between flexor and extensor rats in any of the regions examined. Although there was a tendency of flexor rats to have a higher cortical 5-HTP synthesis rate than extensor rats, this failed to reach statistical significance. These findings are in agreement with the 5-HIAA data (Table 1) which is also believed to provide a measure of 5-HT metabolism and turnover.

TABLE 2

IN VIVO RATE OF TRYPTOPHAN HYDROXYLATION IN THE BRAIN OF FLEXOR AND EXTENSOR RATS

Brain Region	5 HTP Accumulation* ng/g/30 min	
	Flexor	Extensor
Cortex	191.4 ± 18 (7)	154.3 ± 13 (7)
Hippocampus	138.8 ± 12 (7)	148.0 ± 4 (7)
Hypothalamus	298.8 ± 20 (8)	288.4 ± 14 (7)
Midbrain	428.2 ± 22 (8)	417.9 ± 22 (7)
Cerebellum	45.7 ± 4 (7)	47.1 ± 4 (7)
Pons-Medulla	256.1 ± 12 (7)	272.3 ± 29 (7)

*No significant differences between flexor and extensor rats were detected using a two-tailed *t*-test. Rats given 100 mg/kg (IP) NSD-1015 8 days after their last seizure test, and sacrificed 30 minutes later.

DISCUSSION

Flexor rats differ from extensor rats by virtue of their resistance to HLE in the MES test [18]. Buterbaugh [5] has shown that 5-HT depletion will convert a flexor rat to an extensor rat, and conversely that elevations in brain 5-HT will convert extensor rats to flexors. Thus, the HLE response appears to be exquisitely sensitive to manipulations in CNS 5-HT. It does not seem unreasonable, therefore, to propose that the natural resistance of flexor rats to HLE is due to an enhanced serotonergic activity in these rats. In fact, it has been reported that flexor rats have a higher endogenous whole brain 5-HT concentration than extensor rats [4].

The present findings failed to support the hypothesis that the resistance of flexor rats to HLE is due to enhanced serotonergic levels. Indeed, examining 8 different regions of the CNS, we did not find any evidence of elevated endogenous 5-HT levels in flexor rats. Moreover, using 5-HTP accumulation following aromatic L-amino acid decarboxylase inhibition as a measure of 5-HT synthesis rate, we failed to obtain differences between flexor and extensor rats for 6 different regions of the brain.

The reason for the discrepancy between our findings and those which showed a difference in whole brain 5-HT are not readily apparent. However, it does not appear that dissecting the brain into smaller regions is responsible since we also examined whole brain 5-HT in 5 flexor and 4 extensor rats, and failed to observe any difference in steady-state 5-HT levels (flexors = 652.9±26 ng/g vs. extensors = 664.4±13 ng/g). Although differences in 5-HT assays might account for the differences in the two findings, this does not seem likely since we employed a fluorometric procedure [1] very similar to Buterbaugh [4] when we examined whole brain levels, and still failed to detect a significant difference between flexor and extensor rats. One obvious difference between the two studies was the fact that in the present study 3 MES-tests (48 hours apart) were used for the classification of rats into the flexor and extensor categories while the previous study [4] used 4 tests (48 hours apart). However, we have also examined 5-HT and 5-HIAA levels in a small number of rats classified with 4 tests instead of 3 and these also failed to show any differences between flexor and extensor rats. The strain of rat was the same in both studies but differences in the supplier, the time interval between seizure testing and sacrifice, and differences in the post-mortem handling and storage of the tissue which are not available for comparison could have contributed to the disparity in findings.

Although enhanced serotonergic levels were not observed in the present investigation, there is no question that MES-induced HLE is sensitive to manipulations in 5-HT. It may be that 5-HT represents just one of several systems that influence HLE in electroshock-induced tonic seizures, and that this response is regulated by a delicate balance between excitatory and inhibitory systems. Thus, removal of 5-HT can shift that balance in favor of extension. Whatever natural factor has shifted it in favor of non-extension in the flexor rat, it does not appear to be 5-HT and other studies have suggested that it is also not norepinephine [5]. It is interesting to note, however, that factors other than changes in transmitter levels can markedly influence a rat's response to MES. For example, we have found that lesions in the midbrain reticular formation can convert extensor rats to flexor rats [3].

The literature is now replete with studies suggesting that 5-HT exerts seizure attenuating effects (see [2] and [12] for review). However, some investigators have failed to observe this effect [9, 13, 16]. One explanation for these discrepancies appears to be the lack of uniformity in seizure models used by different laboratories. If we examine only those studies that have looked at the tonic components of MES, irrespective of the species, we find consistent evidence that 5-HT exerts an attenuating role [2, 4, 5, 7, 15]. Thus, although elevated 5-HT levels may not be responsible for the resistance of flexor rats to HLE, serotonergic neurons do exert a significant influence on tonic seizures, and it seems important for future studies to be concerned with both how and where this influence occurs.

In conclusion, the present studies show that the natural resistance of flexor rats to MES-induced HLE is not associated with elevated serotonin levels in the regions examined. However, this does not diminish the fact that 5-HT plays an important role in regulating HLE, but rather indicates that other studies will be needed to ascertain the mechanism by which 5-HT exerts its antiextensor effect.

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